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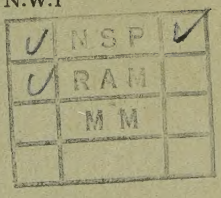

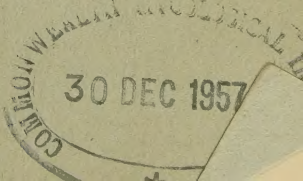

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ON *NEOTTIOSPORA CARICINA* (DESM.) HÖHNEL

By G. J. CUNNELL

Birkbeck College, University of London

(With 4 Text-figures)

An account, based on British material, is given of *Neottiospora caricina* (Desm.) Höhnel with special reference to spore development and to the peculiar structure of the operculate pycnidium. The taxonomy of the species is briefly discussed and the range of 'host' substrata indicated. The behaviour of the fungus in pure culture is described.

Introduction

The genus *Neottiospora* was first described by Desmazières (1843) with *N. caricum* (pl. crypt. no. 1338) as the type species. However, a few years earlier he had published a description of *Sphaeria caricina* (pl. crypt. no. 717) (Desmazières, 1836). In his later publication no reference was made to this species, although, on the printed description which accompanies his exsiccata material of *Neottiospora caricum* in the Kew Herbarium, it is given as a synonym. *S. caricina* is also mentioned as a synonym by Berkeley & Broome (1850) in the first British record of *Neottiospora caricum.*

After examining the type material of both species von Höhnel (1924) came to the conclusion that they belonged to the same fungus. He pointed out that the earlier specific epithet was the valid one and he therefore named this fungus *Neottiospora caricina* (Desm.) Höhnel. My examination of the type material of both species in Kew Herbarium confirms von Höhnel's findings and I have adopted his name.

Occurrence

Desmazières found the fungus in winter and spring in France, and British records confirm that these are the usual seasons for fruiting. I have collected it in a healthy condition between October and March in the south of England although occasionally it can be found up to the middle of May.

Originally Desmazières found it on the dead leaves of *Carex riparia* and *C. pendula* and undoubtedly the former is the commonest host in this country. However, it has been found on the dead leaves of other sedges and also on those of two other marsh Monocotyledons. Collections at the Commonwealth Mycological Institute (excluding those on *C. riparia* and *C. pendula*), are on the following hosts: *C. acutiformis* (I.M.I. 28569), *C. elata* (I.M.I. 21070, 28564), *C. appropinquata* (I.M.I. 17672 *b*) and *Iris pseudacorus* (I.M.I. 21202). A specimen in Herb. Kew. (ex. herb. P. G. M. Rhodes, no. 5293) is on *C. paniculata.* I have found the fungus commonly on

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C. riparia and *C. acutiformis* and on one occasion on the dead submerged leaves of *Typha latifolia*. No doubt this host list could be extended by further collecting in the field. For example, Desmazières records the fungus on *Juncus* sp., although I have not seen a specimen of this, and Saccardo (1898) gives *Carex panicea* as a host.

Pycnidia

The pycnidia are amphigenous but often more numerous on the abaxial side of the leaf. Although completely immersed they appear as conspicuous black dots more or less uniformly scattered along a considerable length of leaf and are not confined to definite patches (Fig. 1 A). They are usually spherical (Fig. 1 C), but sometimes ellipsoidal where the air spaces into which they project are narrow. Mature pycnidia are large, measuring 200–380 μ in diam. (average of 50, 291 μ). Each is firmly attached to the epidermis by the large ostiole region which is 90–200 μ in diam. and has a complex structure. From above (Fig. 1 B) the region is delimited by a black ring 8–12 μ wide which is the uppermost edge of the pycnidial wall and is composed of small, densely packed cells with thick black walls. Within this ring is a narrow translucent zone 6–10 μ thick which surrounds the central lid. The lid is circular, 70–170 μ in diam., black at the edge but lighter in colour centrally, and in vertical section is seen to consist of three layers (Fig. 1 F). The innermost layer is a continuation of the inner pycnidial wall and is formed of thin-walled, almost colourless cells. A few hyphae project from the centre of this layer some distance into the spore mass below. The inner layer grades into the deeper middle layer of small densely packed cells which have thick black walls at the edge of the lid but form a lighter tissue centrally. The outer layer of the lid is composed of the thick cuticle and outer walls of epidermal cells of the leaf. No trace of the hypodermal cells or inner periclinal walls of the epidermal cells can be seen. Stomata may or may not be included in the composition of the lid depending on the position in which the pycnidium is formed.

The thin translucent ring between the lid and the edge of the pycnidium wall is composed of thin-walled, somewhat elongated cells which are orientated obliquely with respect to the leaf surface. By the time the pycnidium is mature the outer walls of the epidermal cells and cuticle above this zone have disappeared. Thus, compared with the surrounding tissues, this ring is mechanically weak and ruptures when spore discharge takes place.

Below the ostiole region the wall is complete, 8–10 μ across, and thin enough for the coloured contents of the pycnidium to be seen through it. The outer layers are composed of densely packed cells with thick brown walls and within this are several layers of flattened, colourless, thin-walled cells (Fig. 1 E). In surface view the cells of the outer layer appear angular, mostly isodiametric, 3–9 μ across (Fig. 1 D). Under the low power of the microscope with transmitted light the crushed pycnidial wall is Light Yellowish Olive* to Yellowish Olive in colour.

* Colour designations according to Ridgway (1912).

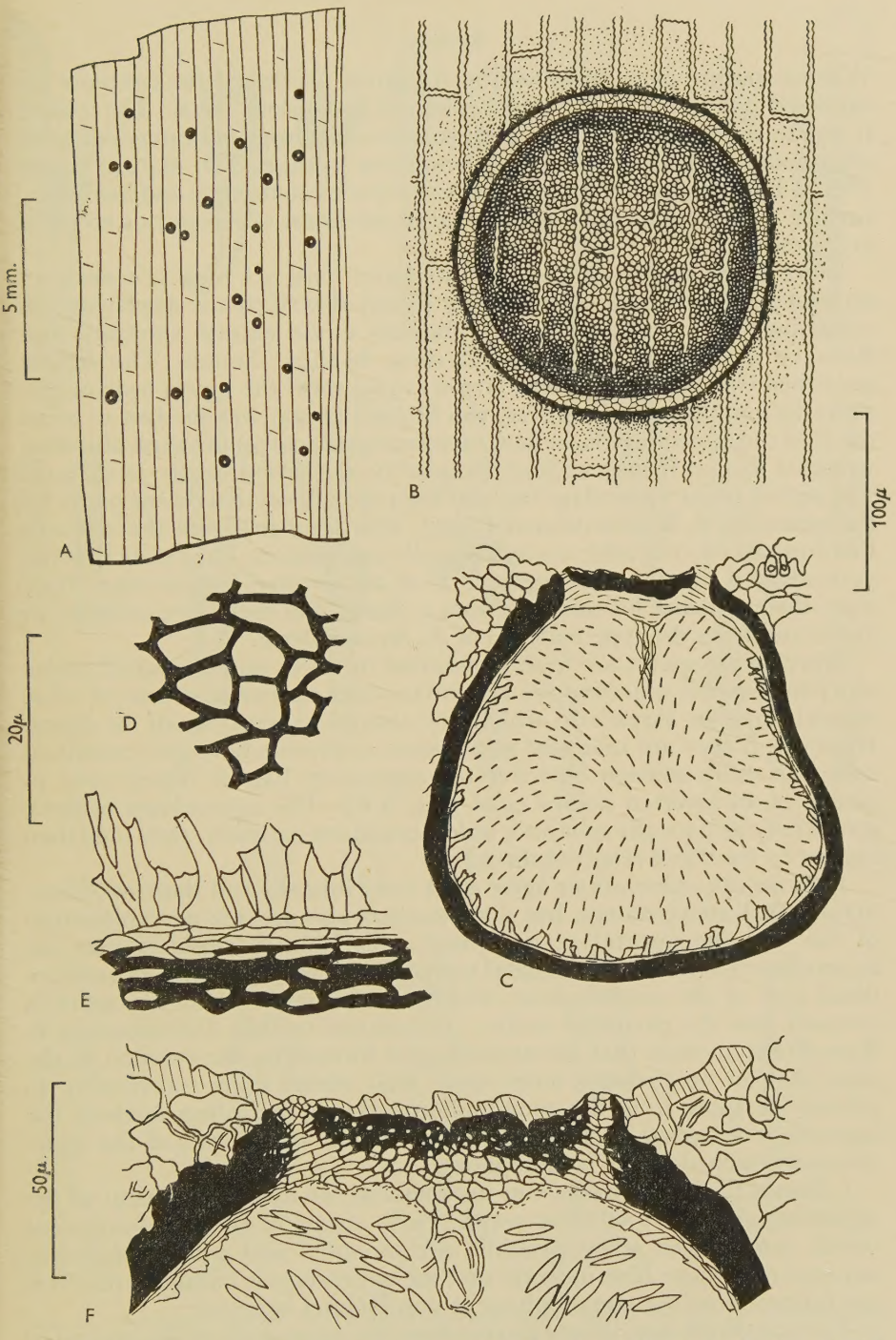

Fig. 1. *Neottiospora caricina*. A, part of *Carex riparia* leaf with pycnidia (×5); B, view of ostiole region of a large pycnidium from above (×250); C, vertical section of small pycnidium stained with gentian violet (×250); D, part of pycnidial wall in surface view (×1325); E, section of pycnidial wall (×1325); F, vertical section of ostiole region stained with gentian violet (×540).

Spores

When a mature pycnidium with its lid intact is crushed the contents are squeezed out as a bright orange-coloured, gelatinous mass. The colour is due to the presence of numerous conspicuous drops of orange-coloured oil among the spores which are themselves hyaline. The mature spores (Figs. 2 K, 3) are mostly straight, naviculoid with acute ends and have thin, smooth walls. Each contains a few large colourless oil drops but no septa are present.

When living spores are mounted in water most are seen to possess an appendage at one end. Desmazières (1843) described the appendage as consisting of '...trois ou quatre filamens d'une tenuité extrême' and Berkeley & Broome (1850) called them hyaline threads. The figures published by Cooke (1871) and Grove (1935) show the spores bearing few short distinct threads. However, von Höhnel (1924) was the first to point out that the appendage is, in fact, membranous, conical in shape but often wrinkled so that it may appear deceptively like several separate threads. The nature of the appendage has also been the subject of a recent paper by Subramanian & Ramakrishnan (1953), who have reviewed the relevant literature but do not refer to von Höhnel's publication. They state that the appendage is mucoid and has the form of an inverted hollow cone. They draw their evidence for this both from a *Neottiospora* they have collected in India and from the type material of *N. caricum* Desm.

When living spores are freshly mounted in water and examined under a dry high-power objective the appendage does appear to consist of a few separate hyaline threads to which are attached several small oil drops. However, if they are mounted in aqueous methylene blue and examined under an oil-immersion objective the appendage can be clearly seen to consist of an inverted hollow cone (Fig. 2 K). The appendages of some spores may be partially split and, unless examined carefully, they may then appear to consist of separate threads.

Thus far my observations agree with von Höhnel's and those of Subramanian & Ramakrishnan, but these workers also provide an explanation of the way the appendage is formed. von Höhnel considers that the appendage is attached to the basal end of the spore and is formed from the distal end of the conidiophore which breaks down when the spore is released into the pycnidial cavity. In contrast to this, Subramanian & Ramakrishnan state that the appendage is formed by the eversion of the distal half of an inelastic outer spore wall which splits transversely to release the spore from the conidiophore. Hence according to them the appendage is apical and the lower part of the outer wall of the spore remains attached to the conidiophore.

I cannot find any evidence in my material for the development of the appendage in either of these ways. Early stages in spore development which can be seen when pycnidia are mounted and gently crushed in aqueous methylene blue indicate that the appendage is basal but that it is not formed from the conidiophore as von Höhnel believed.

The conidiophores, which project from the inner cells of the pycnidial wall, are about 1·5 μ across and up to 20 μ long and become deeply stained

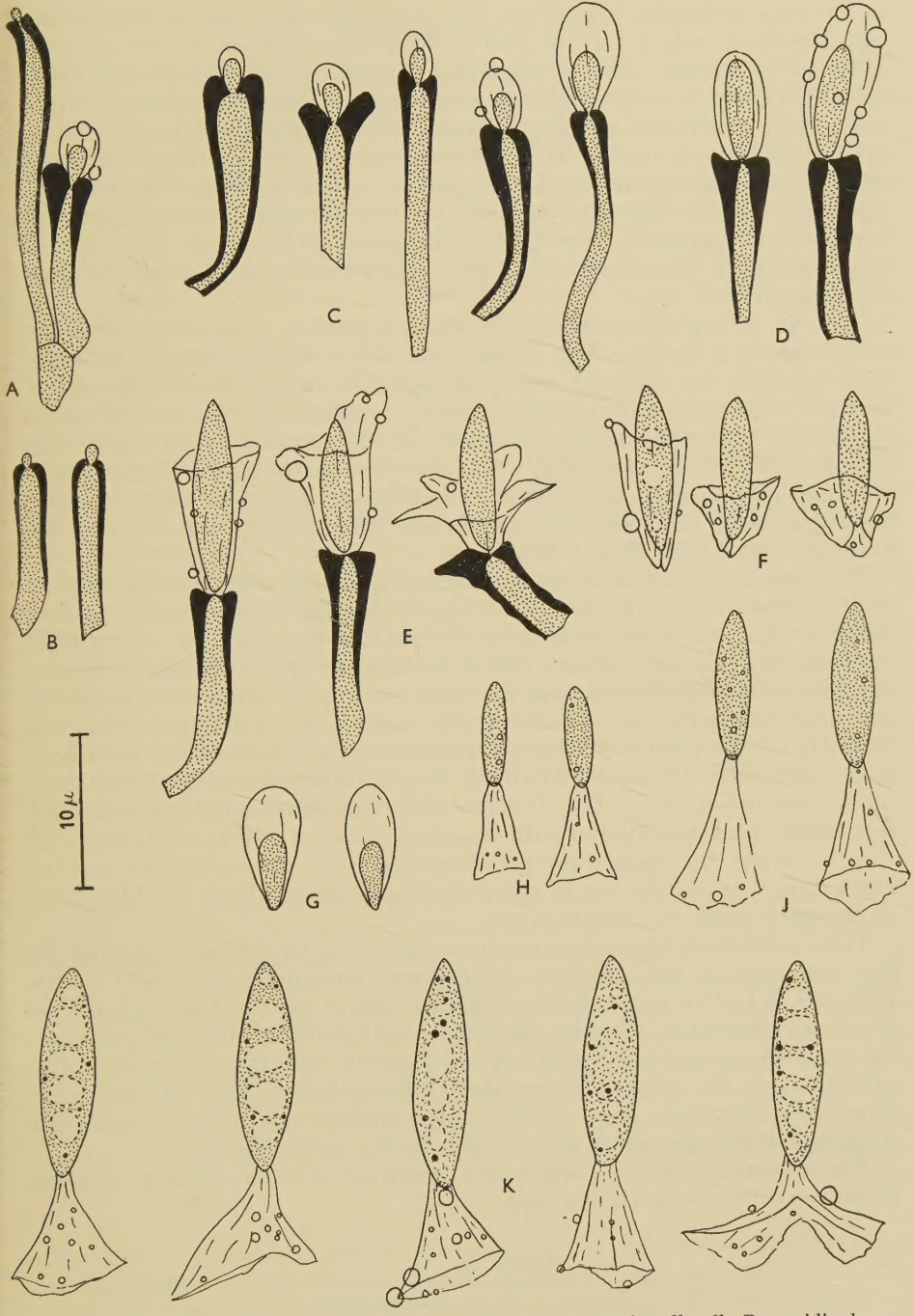

Fig. 2. *Neottiospora caricina.* A, conidiophores attached to basal wall cell; B, conidiophores bearing very young spores; C, conidiophores bearing young spores with thick outer walls; D, older stage of same; E, conidiophores bearing slightly older spores with outer walls ruptured; F, detached spores of similar age showing eversion of outer walls; G, detached spores with intact outer walls; H, young detached spores with everted outer walls forming basal appendages; J, older detached spores; K, five random mature spores. (All from crushed pycnidia mounted in aqueous methylene blue, ×2150.)

in aqueous methylene blue (Figs. 1 E, 2 A). The distal part of the conidiophore is thick-walled and the end is often expanded and deeply concave (Fig. 2 C). At the centre of this distal depression a small spherical bulge appears (Fig. 2 B) which enlarges and becomes elliptical in shape. At an early stage this bulge appears to consist of two parts, a densely staining central region continuous with the cytoplasm of the conidiophore and a colourless outer region crescent-shaped in optical section (Fig. 2 C, D). Later the central region becomes the body of the spore and the colourless area, which is apparently a thick outer mucilaginous wall, gives rise to the appendage. This outer wall is presumably sticky as oil drops are often seen adhering to it.

As the young spore elongates its cytoplasmic connexion with the conidiophore is broken. This seems to be due to the swelling of the outer wall between the body of the spore and the end of the conidiophore. Further enlargement of the spore causes a tension in the outer wall which eventually breaks down or splits near the apex and the torn edges slide down the side of the spore (Fig. 2 E). At this stage the spore, together with the appendage joined to its basal end, becomes detached from the conidiophore (Fig. 2 F) and the appendage becomes everted to form the trailing hollow cone (Fig. 2 H).

The appendages of such young detached spores are often as long or even longer than the spores that bear them and therefore provide further evidence that the outer wall splits at the top and that the whole of it contributes to the appendage. This is contrary to the contention of Subramanian & Ramakrishnan that the outer wall splits about the middle of the spore and only half of it forms the appendage. Further, I have not seen any conidiophores bearing the remaining basal part of the outer wall as they describe. Occasionally young detached spores are found with their outer walls intact (Fig. 2 G). It is probable that these have been dislodged from their weak attachment with the conidiophores during mounting and that normally the spores are not detached until the outer wall is split. Indeed, it may be the act of eversion which brings about the liberation of the spore from the conidiophore.

When detached, the spores are about one-third to one-half their mature size and they undergo further enlargement within the pycnidial cavity (Fig. 2 J, K). The appendages of mature spores usually have several oil drops of various sizes adhering securely to both surfaces of the hollow cone. As noted by other observers the appendages are evanescent and soon disappear when the spores are mounted in lactophenol. They are well preserved if material in good condition is carefully and quickly dried but disappear if the specimens are put into formo-acetic fixatives.

The dimensions of 100 living spores, mounted in water, taken at random from two different collections are:

	Length (μ)			Width (μ)	
Source	Range	Mean	Standard deviation	Range	Mean
Carex riparia	13·0–17·0	14·9	0·72	3·0–5·0	3·8
Typha latifolia	13·0–17·0	14·7	0·79	3·0–5·0	4·1

Herbarium material

The type material of *Neottiospora caricum* Desm. and *Sphaeria caricina* Desm. which I have examined in Herb. Kew. shows all the features of the British material described above. The spores of *Neottiospora caricum* (Fig. 3 A) measure 10·5–16·0 × 2·0–4·0 μ (average of 50, 12·4 × 2·9 μ) and those of *Sphaeria caricina* (Fig. 3 B) 13·0–18·0 × 2·5–4·0 μ (average of 50, 15·1 × 3·2 μ).

The fungus was first recorded in Britain by Berkeley & Broome (1850) and I have examined several of their collections. These agree essentially with my material and the one they presumably refer to in their publication (on dead leaves of Carices, Rudloe, Wilts, coll. C. E. Broome, no date) has spores (Fig. 3 C) 10·5–13·5 × 2·0–3·5 μ (average of 50, 12·0 × 2·7 μ) but most of the appendages have disappeared. They also mention a variety with somewhat larger, olive-coloured spores. The spores of their specimen (Spye Park, Wilts, Jan. 1850 ex. herb. Berkeley) undoubtedly do show this feature, especially in the mass, due to the faintly olive-brown coloured walls. Except for this they are similar to others I have examined (Fig. 3 D) and measure 12·0–17·5 × 2·5–5·0 μ (average of 50, 14·8 × 3·8 μ). They are larger than those of other material collected by Berkeley and Broome but are no bigger than those of my specimens. I have not found any spores with coloured walls among my own collections but those of a specimen collected by E. A. and M. B. Ellis on *Iris pseudacorus* (I.M.I. 21202) are similar to the spores of the variety mentioned by Berkeley & Broome. However, apart from the coloured walls they are similar to other spores I have examined. They bear conspicuous appendages (Fig. 3 E) and measure 11·0–16·0 × 3·0–4·5 μ (average of 50, 13·7 × 3·7 μ).

Presumably shrinkage due to drying accounts for the somewhat lower spore dimensions of the herbarium material compared with those I have found of living spores mounted in water.

Spore discharge

This can be observed quite readily by immersing a mature, dried pycnidium in water. The translucent zone between the lid and pycnidial wall is mechanically weak and at maturity pressure from beneath causes it to rupture suddenly. The lid is pushed off and is followed by a slowly emerging, wide spore tendril (Fig. 4 A). Usually the lid remains firmly attached to the end of the tendril due to the hyphae which project from its undersurface into the spore mass (Fig. 4 B). If damp, living material is immersed in water the tendril soon breaks up due to the dissolution of the mucilage and the spores move rapidly apart. Unlike those of many other fungi, fresh mature pycnidia do not discharge their spores when they are slowly dried.

In culture

The spores germinate slowly in water or on 2 % malt agar at room temperature. A germ-tube usually appears from the distal end first (Fig. 4 C), but if one is formed from the proximal end it arises slightly to the side of, and not directly from, the point where the appendage is attached to the spore.

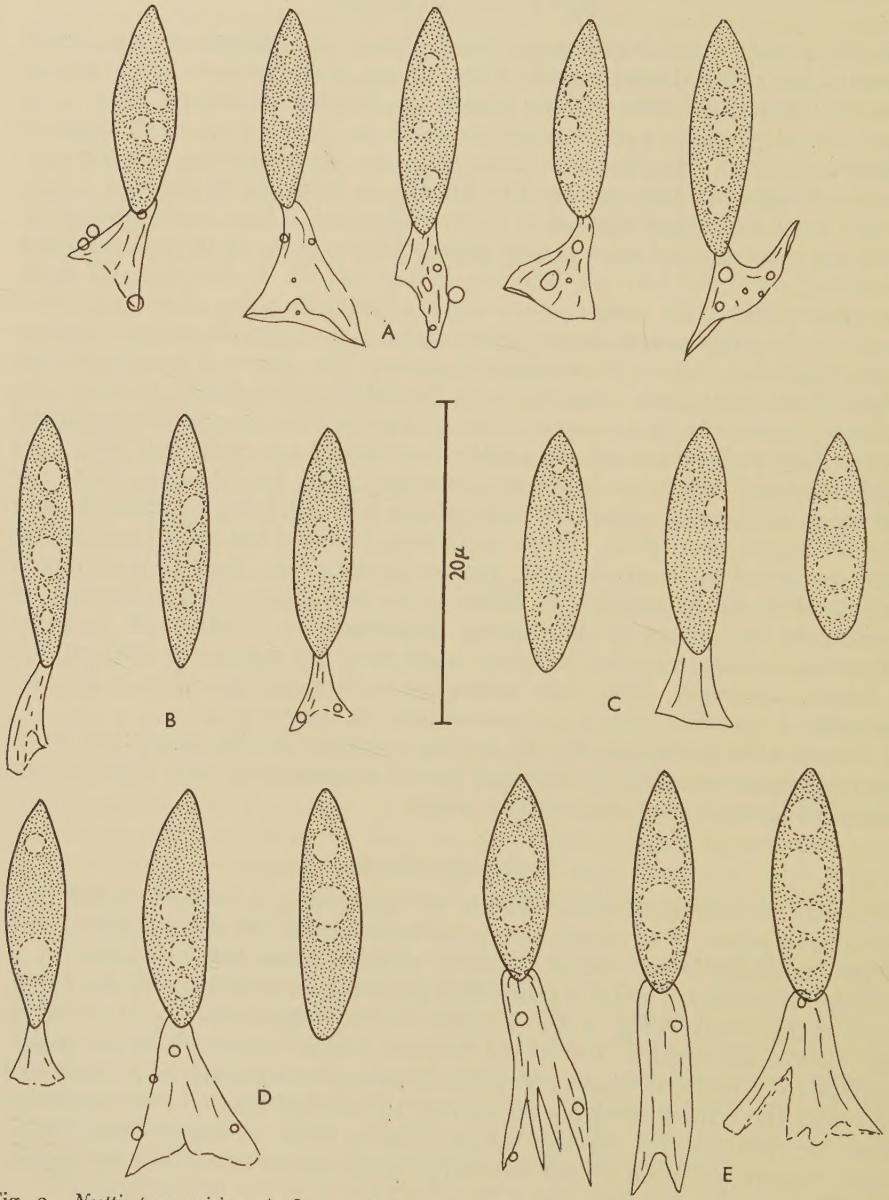

Fig. 3. *Neottiospora caricina*. A, five random spores from type material of *N. caricum* Desm.; B, three random spores from type material of *Sphaeria caricina* Desm.; C, three random spores of British material of *Neottiospora caricum* Desm. (on dead leaves of Carices, Rudloe, Wilts, coll. C. E. Broome, no date); D, three random spores of variety of *N. caricum* Desm. with coloured walls (Spye Park, Wilts, Jan. 1850, ex. herb. Berkeley); E, three random spores of *N. caricum* Desm. from *Iris pseudacorus* (Wheatfen Broad, Norfolk, 27 December 1947, coll. E. A. and M. B. Ellis). A–D from Kew Herbarium, E from Commonwealth Mycological Institute, I.M.I. 21202. (All mounted in aqueous methylene blue, × 2150.)

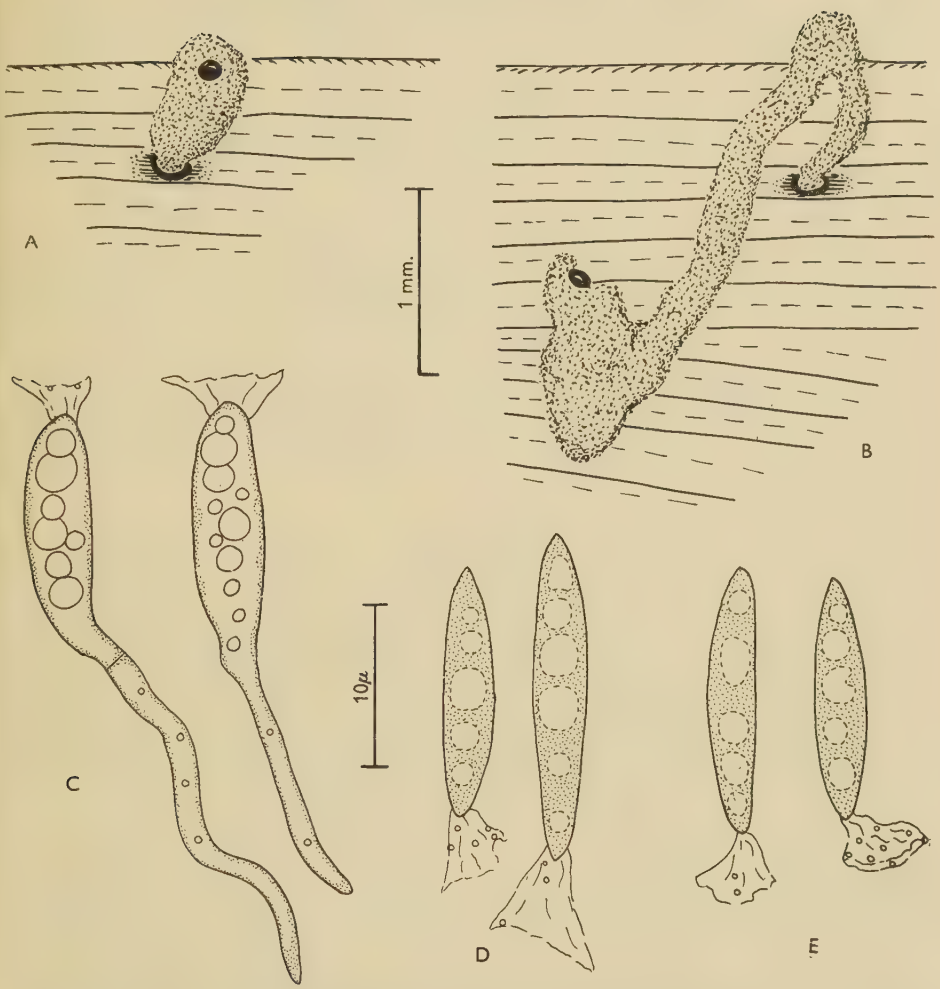

Fig. 4. *Neottiospora caricina*. A, pycnidium on dried piece of *Carex* leaf immersed in water with beginning of spore tendril formed 15 sec. afte rthe lid had been pushed off (×25); B, the same after a further 10 min. (×25); C, germinating spores after 18 hr. on 2% malt agar at room temperature (×2150); D, two spores from pycnidia formed by isolate on 2% malt agar; E, two spores from pycnidia formed on re-infected sterile *Carex* leaves (D and E in aqueous methylene blue, both ×2150).

On 2% malt agar a slow-growing Light Ochraceous Salmon colony is formed which has only a thin surface mycelium. Sectoring is frequent with the production of faster growing regions almost devoid of aerial mycelium. Similar colonies are formed on potato-dextrose agar and oat-meal agar. Fruiting is poor on the agar media used, only a few pycnidia being formed on or near the inoculum. Often the spores (Fig. 4 D) are larger than those found in nature.

On re-infected, sterilized *Carex riparia* leaves a slow-growing, thin, surface mycelium is formed. A few colourless pycnidia appear within the leaf tissue and produce typical, though somewhat large, spores (Fig. 4 E).

Dimensions of 100 living spores taken at random from two different cultures are:

Source	Length (μ) Range	Mean	Standard deviation	Width (μ) Range	Mean
2% malt agar	12·0–20·0	15·2	1·36	2·5–4·5	3·5
Re-infected *Carex*	13·0–18·0	15·6	1·05	3·0–4·5	3·6

Material from my collections has been deposited in the herbaria of the British Museum (Natural History), the Royal Botanic Gardens, Kew, and the Commonwealth Mycological Institute, Kew (I.M.I. 67900 on *Typha latifolia* and I.M.I. 67901 on *Carex riparia*).

I wish to express my gratitude to Prof. C. T. Ingold for his interest and guidance throughout the course of this work. My thanks are due also to Dr R. W. G. Dennis for taxonomic advice and to Dr M. B. Ellis for allowing me to examine his collections.

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STAGONOSPORA SPP. ON *PHRAGMITES COMMUNIS* TRIN.*

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(With Plate 18 and 7 Text-figures)

Stagonospora elegans (Berk.) Sacc. & Trav. and *S. cylindrica* sp.nov. occurring on dead submerged stems of *Phragmites communis* Trin. are described and illustrated. Behaviour of single spore isolates on agar media and on pieces of sterilized *Carex* leaves and *Phragmites* stems is briefly noted.

Introduction

Phragmites communis Trin. is a tall rhizomatous grass often growing in abundance in marshes, at the sides of lakes and on the banks of slow-flowing rivers. It also occurs near the mouths of rivers where the water is distinctly brackish. The erect leafy shoots are annual but in the autumn only the leaf blades fall, the dead, partly sheathed stems remaining in position during the winter and persisting even beyond the following year. Subsequently the culms break, usually at or near the water-level, and the detached pieces drift for a time before they become water-logged and sink. The dead underwater parts often remain attached for several years before they break up.

The two fungi considered in this paper have been found in a healthy condition only on dead submerged stems and can therefore be regarded as regular members of the aquatic fungal flora. They usually occur in clear water and have not been found where mud covers the dead stems with a thick deposit.

Unlike other pycnidial fungi which grow on dead parts of reed-swamp plants (Cunnell, 1956) these two species do not show a marked seasonal periodicity. Presumably this is due to the woody nature of the *Phragmites* culms, which may persist *in situ* for a long time before disintegrating and thus provide a suitable fruiting substratum for these fungi throughout the year.

The procedure in dealing with these fungi is the same as that described in an earlier paper (Cunnell, 1956).

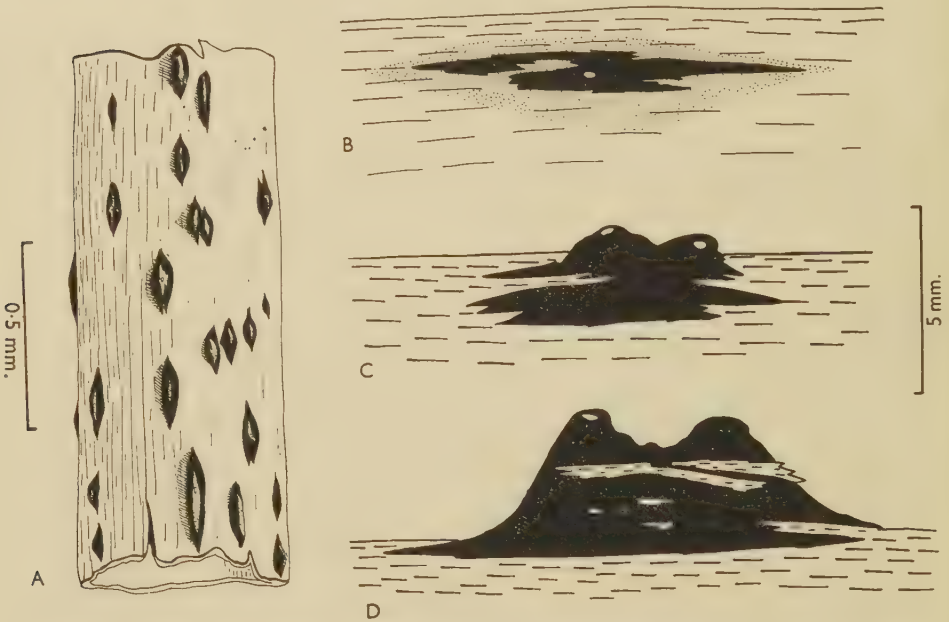
Stagonospora elegans (Berk.) Sacc. & Trav.

This fungus has been found many times, mostly on the lower internodes of *in situ Phragmites* culms, 5–60 cm. below the surface of the water where it is often associated with the aquatic Discomycetes *Belonium excelsius* (Karst.)

* Part of a thesis approved for the degree of Ph.D. in the University of London.

Boud. and *Niptera pulla* (Phill. & Keith.) Boud. (Ingold, 1954). It usually occurs in fresh, clear waters such as Blelham tarn, Lancashire, and Littlesea, Dorset.

The pycnidia are conspicuous, gregarious and more or less evenly distributed along the submerged internodes, giving the stem a black speckled appearance (Text-fig. 1 A). The size and shape of the pycnidia



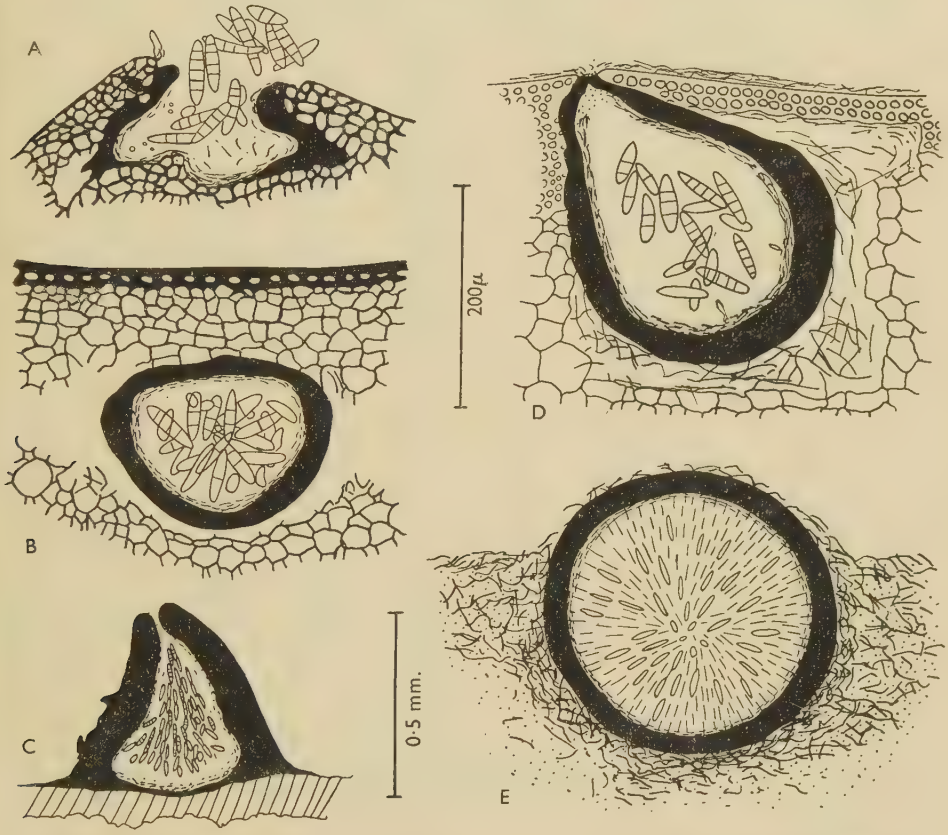
Text-fig. 1. *Stagonospora elegans*. A, part of stem of *Phragmites* with pycnidia (×50); B, single typical pycnidium (×50); C, D, atypical pycnidia, each with two ostioles, from region of stem just above a node (×50).

seem to be affected by the nature of the substratum in which they develop. Typically they are naviculoid and, when fully developed, rupture the outer layers of the stem so that part of the wall can be seen (Text-fig. 1 B). Where the subepidermal layers are softer the pycnidia are not erumpent and only the ostiole region breaks through to the outside. Sometimes, especially just above a node, very erumpent pycnidia are found, each of which may have one or more ostioles borne on a short neck (Text-fig. 1 C, D). Remains of the persistent leaf sheath are often found in this region indicating that the pycnidia, though being initiated in the stem tissue, have grown up through the sheath which has later disappeared.

From the taxonomic viewpoint the completeness of the pycnidial wall has been considered of some importance. However, this again seems to depend upon the degree of lignification of the outer layers of the host stem. Where this is very great the pycnidia are shallow, of irregular shape in section, and have only an ill-defined, basal, spore-bearing layer (Text-fig. 2 A, C). In softer stems the wall is complete and of more or less

uniform thickness and the developing pycnidia may lift the outer layers of the stem away from the inner tissues (Text-fig. 2 B).

Further indication of the truly pycnidial nature of this fungus is found in the structure of the fruit bodies formed in culture. When grown on fairly hard *Phragmites* stems the fruit bodies are shallow and the lower wall is thin or indistinct, but where leaf sheaths are still present the pycnidia are



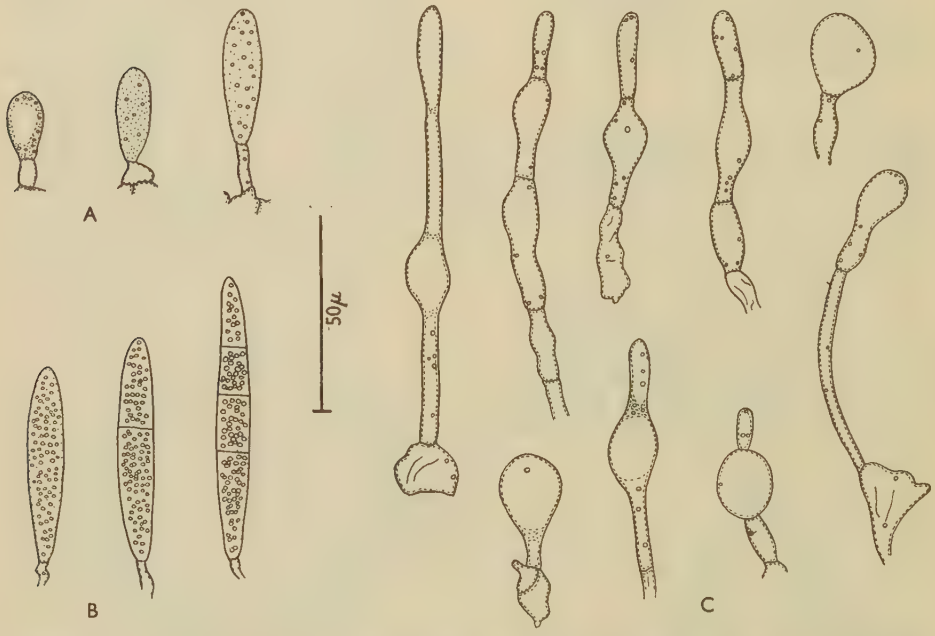
Text-fig. 2. *Stagonospora elegans*. A, vertical section of pycnidium with incomplete wall, formed on a hard *Phragmites* stem (× 150); B, vertical section, not through the ostiole, of a pycnidium in a soft *Phragmites* stem (× 150); C, vertical section of pycnidium shown in Fig. 1 D (× 50); D, vertical section of pycnidium in re-infected leaf-sheath of *Phragmites* (× 150); E, vertical section of pycnidium produced by a colony on oatmeal agar (× 50).

formed within these and have complete walls (Text-fig. 2 D). On sterilized *Carex riparia* leaves the pycnidia are immersed, spherical and have complete walls. If fruiting occurs when the fungus is grown on agar media, the pycnidia are large, half buried in the soft substratum and have complete walls of uniform thickness (Text-fig. 2 E).

Pycnidia from nature on a hard, smooth internode measure 600–1260 × 190–340 μ (average of 50, 849 × 246 μ). The ostioles are variable in size,

being 15–70 μ in diameter, and the wall, where it is exposed, is black, carbonaceous, 18–100 μ thick. The elements of which the wall is composed are so dense and closely packed that their individual limits and shapes cannot be seen, even on crushing.

The young spores are borne on short stalk cells which project from the colourless layer of the flattened, thin-walled cells lining the pycnidial wall (Text-fig. 3 A). They usually become detached from the wall prior to septation and continue to enlarge while embedded in the mucilaginous interior of the pycnidium. Part of the stalk cell may remain attached for a time to the young spore (Text-fig. 3 B).



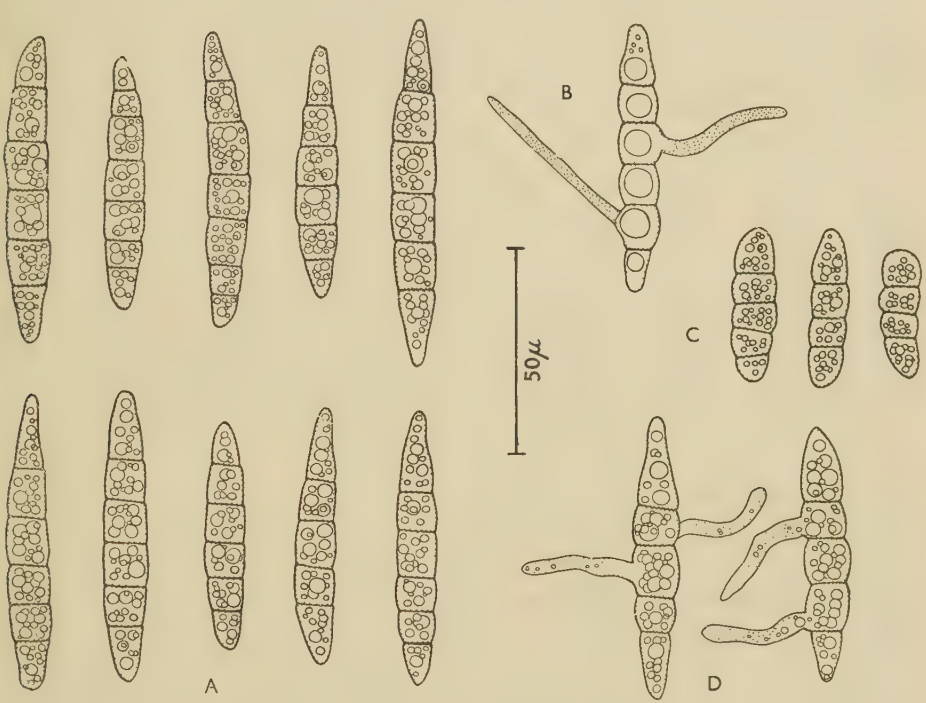
Text-fig. 3. *Stagonospora elegans*. A, stages in the development of young attached spores; B, stages in the development of older detached spores; C, swollen detached cells from a crushed pycnidium. (All × 555.)

When a pycnidium is crushed, especially if not fully developed, numerous short chains of irregular, swollen, highly vacuolated cells are found mixed with the young spores (Text-fig. 3 C). These chains appear to be initially attached to the inner pycnidial wall but later float free or are detached when the pycnidium is crushed.

The mature spores (Pl. 1 A; Text-fig. 4 A) are colourless, large, fusiform and straight or slightly curved. Distally the spore tapers to a rounded end but often the proximal extremity is blunter. (3)4–6 obvious septa are normally present and the smooth lateral walls are constricted at these even in freshly mounted spores. Numerous conspicuous oil drops are present which, in healthy mature spores, are of different sizes.

Dimensions of 100 spores taken at random from two collections are as follows:

Length (μ)			Width (μ)	
Range	Mean	Standard deviation	Range	Mean
55·0–77·0	66·5	5·90	9·0–14·0	11·0
52·0–84·0	65·3	5·30	8·5–12·0	9·9



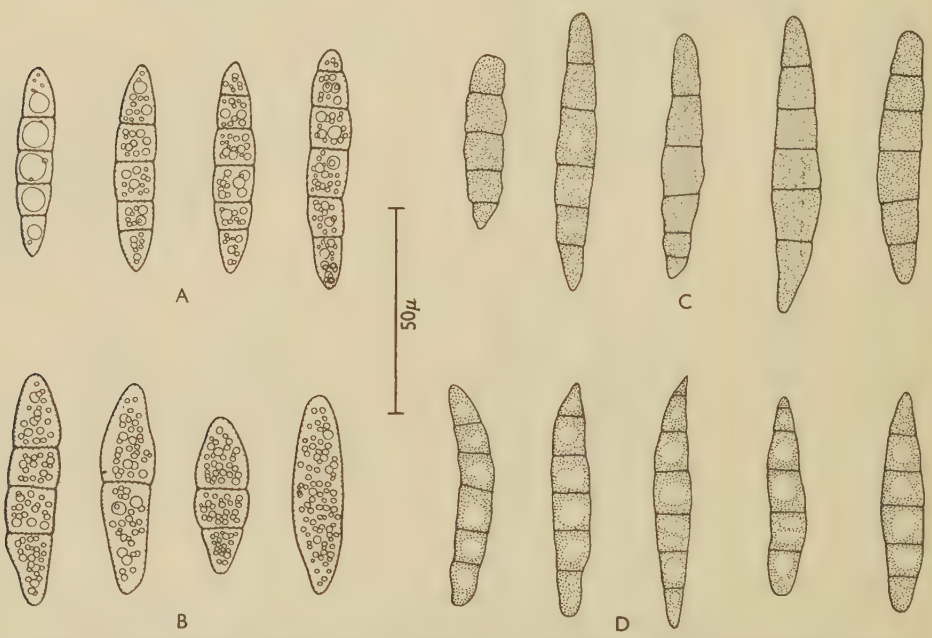
Text-fig. 4. *Stagonospora elegans*. A, ten random living spores from nature; B, germinating spore, from old closed pycnidium, after 8 hr. in water at room temperature; C, atypical short spores from nature; D, typical spores from nature germinating after 8 hr. in water at room temperature. (All ×555.)

Occasionally a pycnidium is found containing short spores with few septa and blunt ends (Text-fig. 4 C). Rarely, in pycnidia which have been exposed due to an abnormal fall in water-level, and also in old, unopened pycnidia, some spores are found with the oil coalesced to a single drop in each cell. These uniguttulate spores are as viable as typical ones (Text-fig. 4 B).

Grove (1935) suggests in a footnote that this fungus may not be a *Stagonospora* because the pycnidium is often incomplete. However, as pointed out above, the extent and thickness of the wall is greatly affected by the hardness of the substratum. Where this is soft the wall is complete and there is no reason to doubt that this fungus is, in fact, a species of *Stagonospora*.

At least twelve *Stagonospora* spp. have been recorded on *Phragmites* but the majority can be excluded from further consideration here due to their spore sizes and septation.

The spores of *S. vexata* Sacc. (Saccardo, 1884), originally cited as *Hendersonia arundinacea* Sacc. non Desm. (Saccardo, 1879), are described as 60–70 μ, 10- to 12-septate. These are narrower and have more septa than the spores of my material, which cannot therefore belong to this species. However, a variety has been described from Finland, *Stagonospora vexata* v. *pauperior* (Karst.) Sacc. (Saccardo, 1884), with 5- to 7-septate spores measuring 65–75 × 9–10 μ. I have not been able to see type or other named material of this but it seems to be very similar to my species.



Text-fig. 5. *Stagonospora elegans* A–C; *S. dolosa* D. A, four random living spores from pycnidia formed in a re-infected leaf-sheath of *Phragmites*; B, four random living spores from pycnidia of an old culture on potato-dextrose agar; C, five random spores of type material of *S. elegans* (Berk.) Sacc. from Herb. Kew. mounted in lactophenol with cotton-blue; D, five random spores of *S. dolosa* (Sacc. & Roum.) Sacc. (coll. Prof. Gwynne-Vaughan, Nahultara, Co. Galway, 1 August 1911) from Herb. Kew. (All × 555.)

S. dolosa (Sacc. & Roum.) Sacc. with 5-septate spores measuring 60–70 × 10 μ, was first described from France (Saccardo, 1882*b*). The pycnidia of the small amount of type material in the Kew Herbarium are similar to those of my material but I have been unable to find any spores. However, I have examined Irish material of this species (coll. Prof. Gwynne-Vaughan, Lough Nahultara, Co. Galway, 1 August 1911). This is identical with my fungus in every respect and is of interest in having the same erumpent pycnidia, often with two or three ostioles, where parts of the close-fitting leaf sheath remain. The spores (Text-fig. 5 D)

measure 48·0–67·0 × 8·5–11·0 μ (average of 50, 57·2 × 9·7 μ) and have (4) 5 (6) septa.

Berkeley (1841) was the first to establish the genus *Hendersonia*, which he based on *H. elegans* as the type species. He described the fungus as having '. . . long broadly fusiform pedunculate colourless spores, with from 6–8 dissepiments. Articulations sometimes swollen, often quite even; each of the central ones containing a single large globose nucleus, with occasionally a few granules. Some of the spores are abortive.' Saccardo (1882*a*) divided the genus into *Hendersonia* with coloured spores and *Stagonospora* with colourless ones and later Berkeley's species was transferred to the latter genus (Saccardo, 1911, 1913).

I have examined the type material of Berkeley's fungus in the Kew Herbarium (from Tansor, Norths. April 1838). The whole specimen consists of a piece of *Phragmites* stem on which are about fifteen pycnidia. These are as Berkeley described and are only erumpent in the ostiole region. There was insufficient material to be sectioned but a single pycnidium has yielded spores in excellent condition (Text-fig. 5 C). They are embedded in mucilage containing a few oil drops and agree with Berkeley's description though the single nucleus (presumably an oil drop) per cell is not apparent. As pointed out previously (Cunnell, 1956) the presence of a single large oil drop in each cell is a common feature of old or dead spores of these fungi and is often found in spores of herbarium material. However, Berkeley's specimen is well preserved and the spores examined from the closed pycnidium have more nearly retained the appearance they presumably had when they were alive. Mounted in lactophenol with cotton-blue the positions of the numerous small oil drops can be seen as light, round areas in the stained cytoplasm.

In shape and septation the spores agree with Berkeley's description and illustration but the abortive spores he refers to are almost certainly young detached ones. He gives no spore measurements in his description but the dimensions of random spores from the single pycnidium that I have examined are: 42·0–73·0 × 8·0–11·0 μ (average of 50, 56·4 × 9·3 μ). They are somewhat smaller than the living spores that I have measured but the ranges overlap to a great extent and the difference may well be due to shrinkage in the dried herbarium material. The spores are (3) 4- to 6-septate as in my collections.

There can be little doubt that the fungus that I have found on *Phragmites* is the same as Berkeley's and I have followed Saccardo (1911, 1913) and Grove (1935) in using his specific epithet. Probably *S. dolosa* (Sacc. & Roum.) Sacc. is the same species but my examination of the type material is not sufficiently satisfactory to make this point certain. *S. vexata* v. *pauperior* (Karst.) Sacc. may also be the same fungus but as I have been unable to examine the type material a definite conclusion cannot be reached.

The spores germinate readily in water at room temperature. The lateral walls bulge and a single germ-tube is produced from each cell. Usually germ-tubes are formed by the non-terminal cells first (Text-fig. 4 D).

The young colonies on 2 % malt agar are circular, non-staling and have a uniform edge formed by widely spaced leading hyphae (Pl. 18 B). The

aerial mycelium is uniform, cottony and white. The reverse is white though it may be Light Olive Grey* centrally. Colonies on potato-dextrose agar and oatmeal agar are similar, though the reverse is Naples Yellow in some isolates. The growth rate is slightly less on oatmeal agar than on the other media used.

The aerial mycelium of old colonies on all three media is dense, cottony, Light Olive Grey to Dark Olive Grey. Vigorous growth results in the sides of the Petri dishes becoming covered by a close mass of mycelium which also extends on to the lid. Where this occurs dense, black, sclerotial masses are formed on the sides of the dishes. In some isolates humps of Deep Olive Grey mycelium are also formed which rise above the uniform surface of the aerial mycelium. Many glistening 'transpiration' drops are apparent on the aerial hyphae, probably due to the fact that the air gap between the bottom and lid of the Petri dish is sealed by the extensive mycelium and evaporation is prevented. The reverse is Mouse Grey to Blackish Mouse Grey.

Fruiting on the three media is variable but never great and an isolate rapidly loses its capacity to sporulate at all. The spores produced in culture are much shorter, though wider, than those found in nature (Text-fig. 5 B) and fewer septa are present.

A white cottony surface mycelium is formed when the fungus is grown on sterilized pieces of *Phragmites* stem. When the culms used are hard only a few superficial and incomplete pycnidia are formed after 3 months. On soft pieces, and where leaf sheaths are present pycnidia appear, mostly below water-level, after about 8 weeks. As already indicated the completeness of the lower part of the pycnidial wall depends on the hardness of the substratum and it is only uniformly thick when the pycnidium is formed within the leaf-sheath tissue. If left submerged for a long time the pycnidia develop definite necks similar to those sometimes found in specimens from nature. The spores present are typical in appearance but are much smaller than naturally occurring ones (Text-fig. 5 A).

Complete, spherical pycnidia are formed on the submerged parts of sterilized *Carex riparia* leaves. The spores are similar to those from pycnidia formed on re-infected *Phragmites* leaf sheaths.

Dimensions of 100 random spores taken from three different cultures are:

	Length (μ)			Width (μ)		
Source	Range	Mean	Standard deviation	Range	Mean	Septa
Potato-dextrose agar	37·0–58·0	47·2	4·65	11·0–16·0	13·1	0–5
Re-infected *Phragmites*	46·0–66·0	53·6	3·83	8·0–11·0	9·3	3–6
Re-infected *Carex*	41·0–63·0	50·8	5·09	8·5–11·5	10·1	4–6

Specimens of this species have been deposited at the Commonwealth Mycological Institute (Herb. I.M.I. 63876), Herb. Kew. and the British Museum (Natural History).

* All colour designations according to Ridgway (1912).

Stagonospora cylindrica sp.nov. (Plate 18 C, D, Text-figs. 6, 7.)

Pycnidia dispersa, nigra, immersa, ellipsoide, magna 590–1020 × 200–400 μ, papillata, poro 35–70 μ transverso. Sporulae cylindricae, plerumque rectae, utrinque rotundae, 3 (4)-septatae, non constrictae ad septa, aut una olei gutta magna aut duabus, paucisque parvis praesentibus in unaquaque cella, hyalinae, 48–78 × 8·0–11·5 μ.

Hab. In caulibus emortuis submersis *Phragmitis communis*, circa Flatford Mill, Essex, in Anglia, 27 September 1953.

Typus: Herb. I.M.I. 63875.

This fungus has been found on *Phragmites* in a somewhat brackish cutting off the River Stour near Flatford Mill, Suffolk, and in freshwater gravel pits at Bedfont and Staines, Middlesex. It has always been found on submerged, rather soft *in situ* stems 5–75 cm. below water-level.

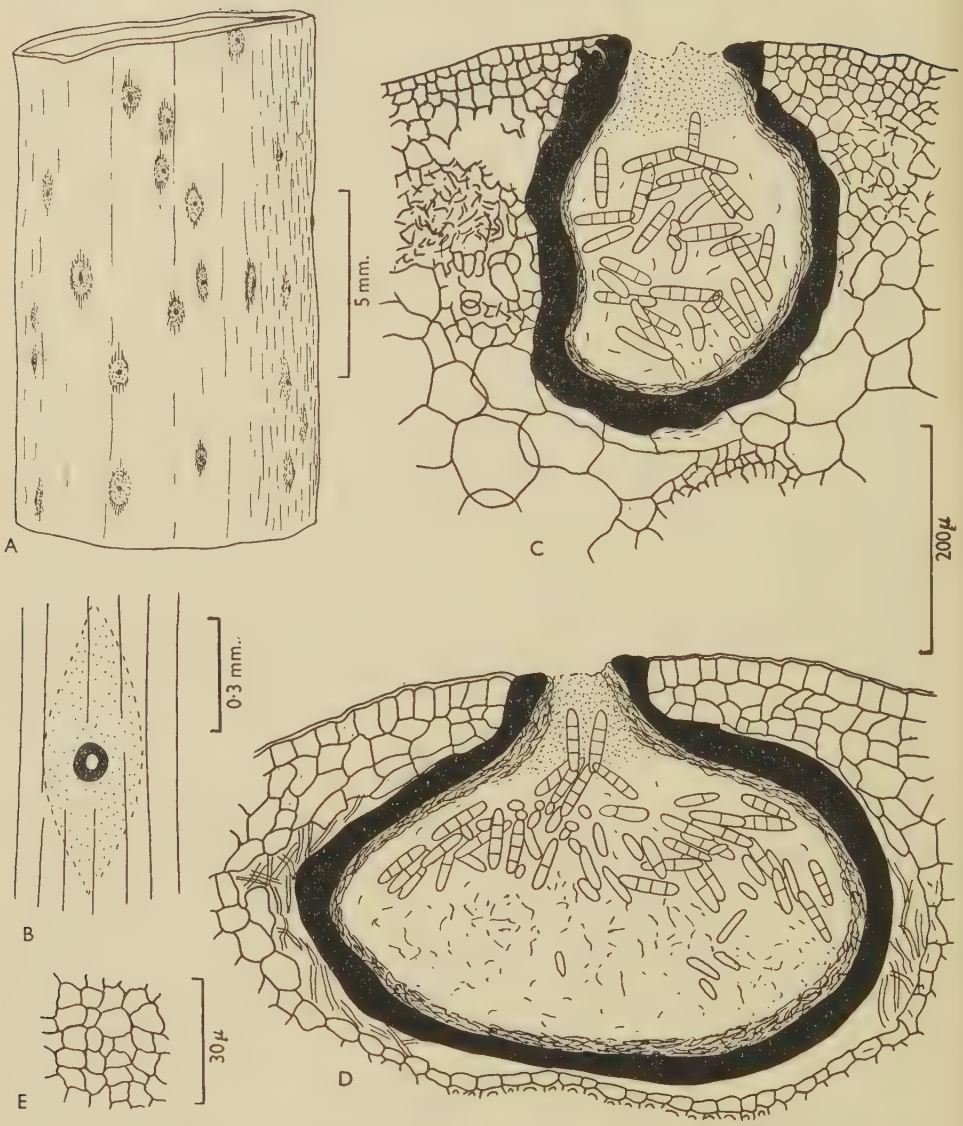
The pycnidia (Text-fig. 6A) are more or less evenly scattered and are not confined to distinct patches. Unlike those of *Stagonospora elegans* the pycnidia are completely immersed though the ostiole region may be slightly raised where the outer tissues of the stem are hard. The epidermis of the stem is rarely torn and the position of the pycnidium is only indicated by a brown, elongated spot around the ostiole (Text-fig. 6 B). The latter is large, 35–70 μ in diameter.

It has been shown that the anatomy of the stem of *Phragmites* varies considerably, especially with regard to the air cavities which may be present in the outer layers between the outer ring of small vascular bundles (Stant, 1953). In the Flatford Mill material there are large, clearly defined air cavities. The pycnidia (Text-fig. 6 D) lie in these where they have been able to expand during development, and, in consequence, are large, ellipsoidal and measure 590–1020 × 200–400 μ (average of 50, 760–309 μ). However, the stems of *Phragmites* from Staines have only small patches of thin-walled cells between the outer bundles, or small cavities resulting from the breakdown of these. Here the pycnidial walls are more irregular in outline and the pycnidia (Text-fig. 6 C) are smaller, measuring 430–850 × 200–350 μ (average of 50, 654 × 263 μ).

The pycnidial wall is black, 17–35 μ thick and is complete whether the pycnidium is situated within an air cavity or is surrounded by crushed tissues of the host. The outer layer of the wall is composed of small, densely packed cells (Text-fig. 6 E).

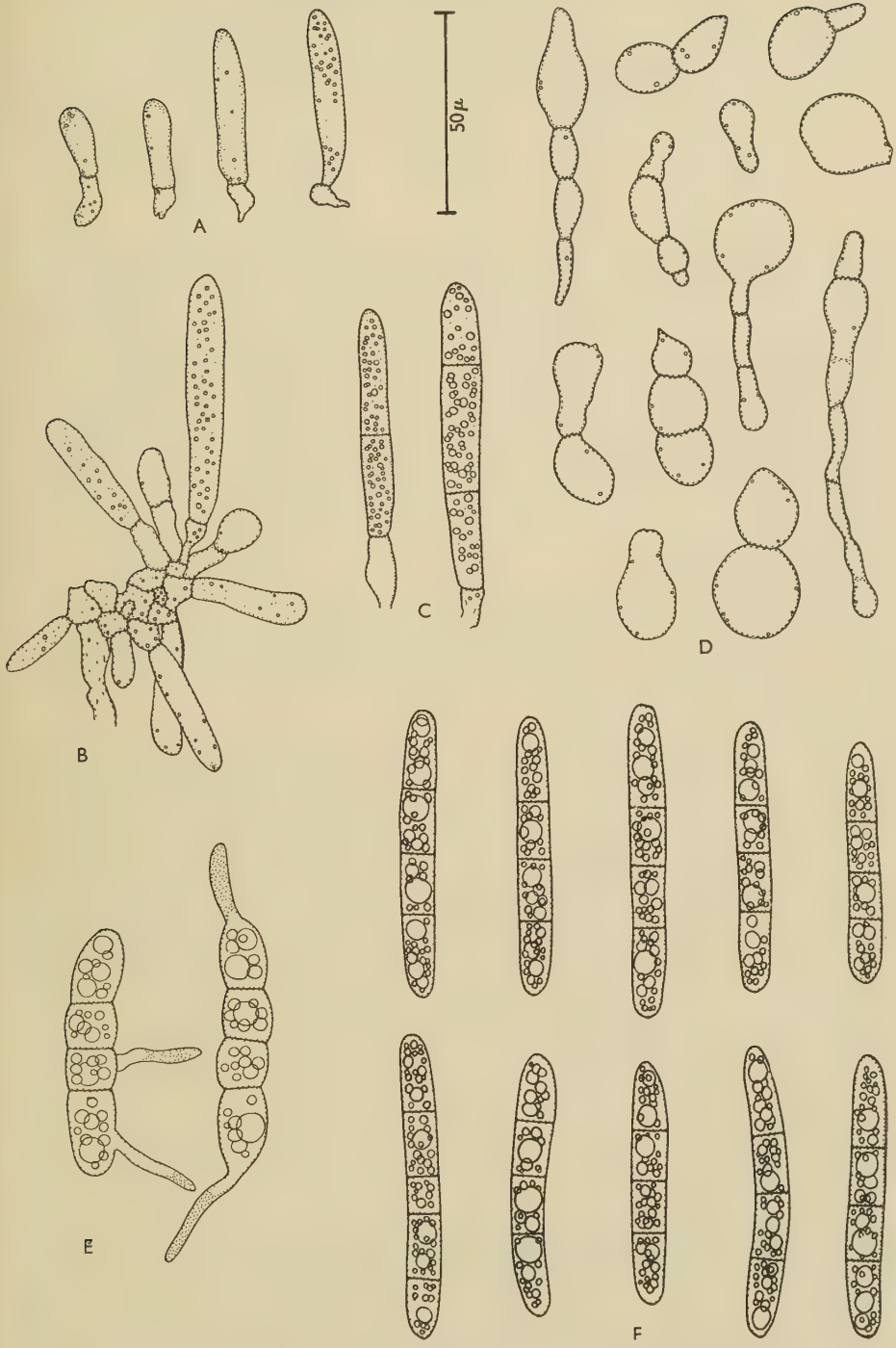
The inner lining of the pycnidium consists of several layers of thin-walled, flattened cells. The spores are borne on short, irregular cells of this layer (Text-fig. 7 A, B). Sometimes they become free before septation begins but attached septate spores have also been found (Text-fig. 7 C). Intermingled with the young attached spores are numerous variously-shaped, thin-walled, vacuolated cells which may be single or in short chains (Text-fig. 7 B). When a pycnidium is crushed most of the smaller of these cells remain attached to the wall but the larger, globular ones float free (Text-fig. 7 D). A few are found among the mass of mature spores but most occur beneath them in the basal region of the pycnidial cavity. They form a lightly stained zone devoid of mature spores in sections mounted in lactophenol with cotton-blue (Text-fig. 6 D).

The spores (Pl. 18 C; Text-fig. 7 F) are colourless, large, mostly straight,



Text-fig. 6. *Stagonospora cylindrica*. A, part of stem of *Phragmites* with pycnidia (× 5); B, single pycnidium on same (× 50); C, vertical section of pycnidium growing on *Phragmites* stem which has no definite air cavities (× 150); D, vertical section of pycnidium growing on a *Phragmites* stem which has large air cavities (× 150); E, part of crushed pycnidial wall (× 555).

rarely curved and have rounded ends with the proximal one sometimes recognizable due to the blunt point of attachment. Three, rarely four, obvious septa are present and in freshly mounted spores the smooth walls are not constricted, although after a short time in water they appear so due to expansion (Pl. 18 C). Numerous oil drops of various sizes are present



Text-fig. 7. *Stagonospora cylindrica*. A, young attached, non-septate spores; B, part of inner pycnidial wall with young attached spores and globular cells; C, young, attached spores with septa; D, swollen detached cells from the cavity of a crushed pycnidium; E, germinating spores after 12 hr. in water at room temperature; F, ten random living spores from nature. (All mounted in water, × 555.)

but it is a regular feature that in mature spores each cell contains one or two much larger than the rest.

Dimensions of 100 spores taken at random from two collections are:

Length (μ)			Width (μ)	
Range	Mean	Standard deviation	Range	Mean
55·0–77·0	66·9	4·20	8·0–10·0	8·5
48·0–78·0	67·2	4·87	8·5–11·5	9·8

The spore sizes of this fungus are very similar to those of *Stagonospora elegans* and, as pointed out in the previous section dealing with that species, nine out of the twelve *Stagonospora* spp. recorded on *Phragmites* have much smaller spores and need not be further considered here. *Stagonospora vexata* Sacc. (Saccardo, 1884) is stated to have 10- to 12-septate spores and can therefore also be excluded. Clearly this fungus is not *S. elegans*, the spores of which, though similar in size, are fusiform and mostly 5-septate. Further, although the two fungi produce similar colonies on agar media, the growth rates are very different. A 5-day-old colony of *S. elegans* (Pl. 18 B) is as large as a 10-day-old colony of this fungus (Pl. 18 D) grown under the same conditions.

Therefore it appears that this is a species not previously recorded on *Phragmites* though the possibility remains that it may have been described from another host. Many *Stagonospora* spp. have been recorded on members of the Gramineae but I have not been able to find a description among these that could possibly apply to this fungus and, therefore, it has been described as a new species.

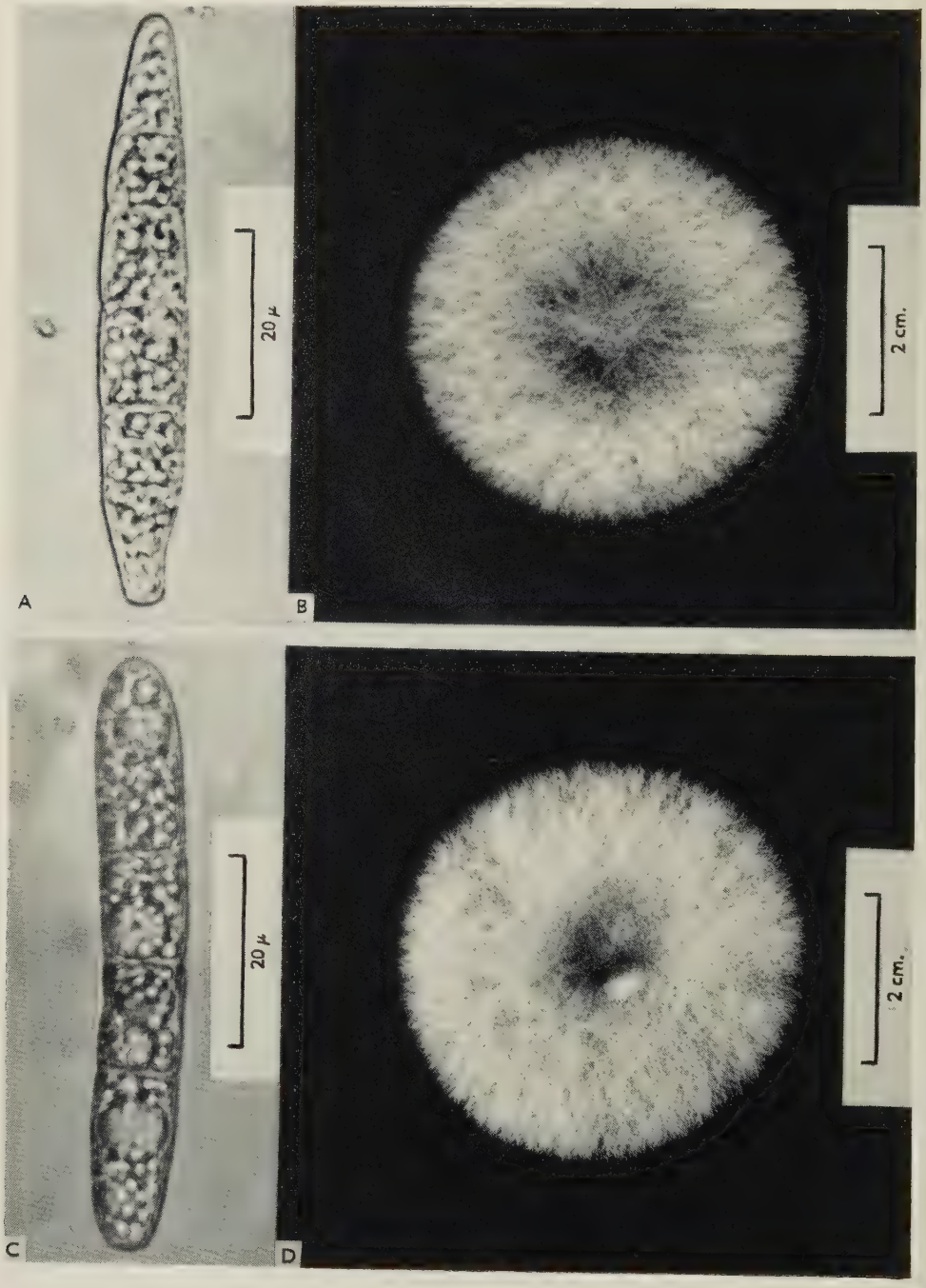
The spores germinate readily in water at room temperature (Text-fig. 7 E). The lateral walls bulge and germ tubes are produced by each cell. Often the terminal cells form germ tubes first but there appears to be no regular order of germination of the cells of each spore.

On 2 % malt agar the colonies are circular, non-staling and have an even edge of widely spaced leading hyphae (Pl. 18 D). The aerial mycelium is loosely cottony, Castor Grey centrally grading to white at the margin. The reverse has a black central region surrounded by a zone of black dots which are small knots of dark brown hyphae formed against the bottom of the dish. On potato-dextrose agar the growth rate is greater but otherwise the colonies are similar. On oatmeal agar the growth rate is less and the aerial mycelium is much denser but the reverse is similar to that of colonies grown on malt agar.

The aerial mycelium of old colonies on all three media is uniform, cottony and Pale Olive Grey to Dark Olive Grey. The reverse is black due to the many knots of dark brown hyphae formed against the bottom of the dish.

On sterilized pieces of *Phragmites* stem a thin surface mycelium is formed and a few pycnidia containing typical spores may appear after about four months. When grown on sterilized *Carex* leaves no pycnidia have been formed.

Material of this species has also been deposited in Kew Herbarium and the British Museum (Natural History).



(*Facing p.* 455)

My sincere thanks are due to Prof. C. T. Ingold for his encouragement and guidance during the course of this work. I also wish to thank Prof. E. H. Warmington for checking the Latin diagnosis.

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EXPLANATION OF PLATE 18

A, B. *Stagonospora elegans*. A, single living spore mounted in water (× 1350); B, 5-day-old culture grown on 2 % malt agar at 23° C. in the dark (× 1·2).
C, D. *S. cylindrica*. C, single living spore mounted in water (× 1350). D, 10-day-old culture grown on 2 % malt agar at 23° C. in the dark (× 1·2).

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POLYPORUS LIGNOSUS KLOTZSCH AND ITS IDENTITY

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(With Plate 19 and 1 Text-figure)

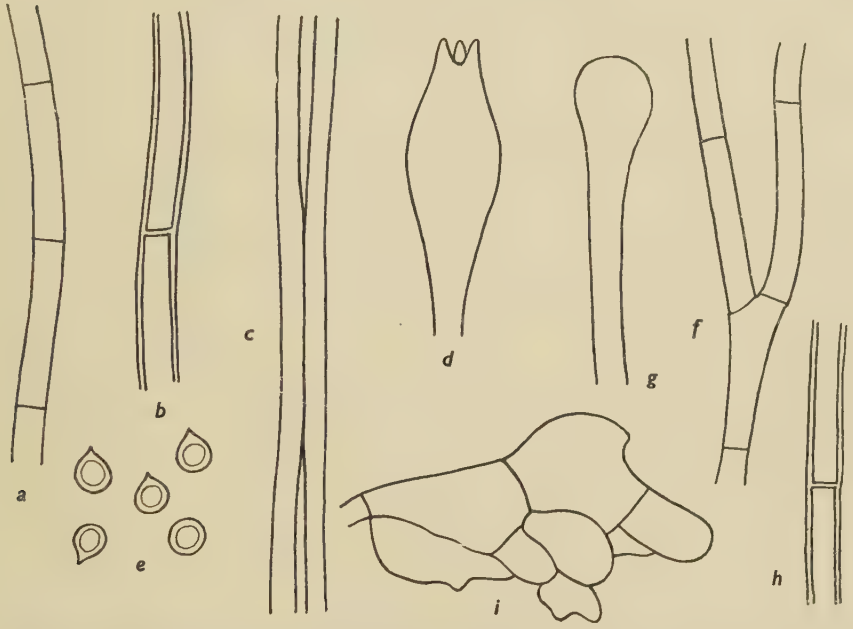
Polyporus lignosus is often confused with *P. zonalis*. They are two distinct species. Besides other characters, *P. lignosus* is usually parasitic while *P. zonalis* is commonly saprophytic. The two fungi are distinguished by their cultural characters. While rhizomorphic strands develop in *P. lignosus* as in nature, such strands are absent in *P. zonalis*, both in nature and in culture. Further, *P. lignosus*, with its thin-walled hyphae with cross-walls, can be distinguished from *P. zonalis*, in which the hyphae are very thick-walled and contain no visible cross-walls. No hyphal fusions occur when *P. lignosus* and *P. zonalis* are paired in culture.

Polyporus lignosus Klotzsch, which is also commonly known as *Fomes lignosus* (Klotzsch) Sacc., is often confused with *Polyporus zonalis* Berk. An attempt is made here to clear up this confusion by describing sporophores of *P. lignosus* along with its cultural characters. The cultural tests laid down by Bavendamm (1928) and Preston & Mclennan (1948) have been followed in determining the nature of the rot. Colour nomenclature is that of Ridgway (1912).

Occurrence. In Malaya and in Ceylon, *P. lignosus* is known as the cause of the commonest root disease of *Hevea*. Young (1954) has also recorded it for South India, Java, Sumatra, Borneo, East and West Africa, the Belgian Congo, and in the Amazon Basin in South America. The disease occurs in a great variety of plants and is relatively common. It has been recorded on *Hevea*, cacao, coffee, tea, coconut, oil palm, various species of *Ficus*, jack, mango, bamboo, manioc, camphor, and on numerous leguminous and other plants used for green cover or shade such as *Erythrina*, *Albizzia*, *Leucaena glauca*, *Tephrosia candida* and *Crotalaria*. Bose (1921) collected it from Darjeeling (Bengal) in 1921 and from Tocklai Tea Station (Assam) in 1940. Recently, he has kept the fungus growing in the form of strands and thin mats on the outer surface of the roots of infected specimens of *Hevea brasiliensis* obtained from Rubber Research Institute of Ceylon. These strands are really the rhizomorphs, which are concerned in the transport of food from the 'food base' for some distance, as held by Young (1954) in connexion with his study of white root disease of *Hevea*.

In the present study, the fungus was collected by the junior author on living *Ficus bengalensis* and *Bauhinia purpurea* in Dehra Dun district. In *B. purpurea*, the sporophores develop at the base of the tree (Pl. 1, fig. 1). Only the heartwood is attacked. Once the fungus was collected on an ascu-treated *Shorea robusta* (sál) pole.

Sporophore. Fruit bodies sessile to effuso-reflexed, dimidiate to flabelliform, usually imbricate (Pl. 19, fig. 1), leathery when fresh, hard and rigid when dry, margin thin, entire, strongly incurved on drying; upper surface glabrous, applanate when fresh, convex on drying, concentrically zoned, with fine radiating wrinkles when dry, reddish brown (Light Ochraceous Buff to Pinkish Buff with darker zones of Cinnamon to Chestnut Brown); context 0·5–3·5 mm. thick, white or light coloured,



Text-fig. 1. *Polyporus lignosus. a*, context hypha, thin-walled; *b* and *c*, context hyphae, thick-walled; *d*, basidium; *e*, basidiospore; *f*, culture hypha, thin-walled; *g*, swelling on hypha; *h*, culture hypha, thick-walled; *i*, cuticular cells.

fibrous, fibres running more or less parallel to the surface, hyphae hyaline, thin to thick-walled, thin-walled with many cross-walls and thick-walled with a few cross-walls (Text-fig. 1 *a*, *b*), sometimes lumen almost obliterated, unbranched (Text-fig. 1 *c*), 3·6–7·3 μ broad; hymenial surface pink (Pale Ochraceous Salmon to Pinkish Buff), smooth, glossy with silky sheen, pores 7–9 per mm., round to oval, or angular, pore tubes in one layer, Pinkish Buff, nearly concolorous with the context, 1–2·5 mm. long; basidia pyriform (Text-fig. 1 *d*), 12·4–20·4 × 7·3–11·6 μ; spores hyaline or nearly so, thin-walled, smooth, subglobose to globose, apiculate, 1-guttulate (Text-fig. 1 *e*), 3·6 × 4·6 μ in diameter.

Rot. The fungus causes white pocket rot in the heartwood of living trees. The pockets are 5–10 mm. or more long, 2·5 mm. broad, delimited from one another by pale reddish-brown lines (Pl. 19, fig. 2) in the form of large reticulations. In late stages of decay, the tissues in the pockets become fibrous.

Cultural characters (*on malt agar at* 25° C. *in the dark*)

(*a*) *Growth characters*. Growth moderately rapid, radial growth 3·0 cm. at 25° C. in 7 days. Advancing zone white, aerial mycelium extended up to the periphery in the form of separate strands, mat white, zonate, silky with radiating tufts of aerial mycelium raised in the form of rhizomorphs at the periphery (Pl. 19, fig. 3), such mycelium condensed to form white irregular lines over the mat. Fan-shaped hyphal strands formed on the walls of the plate. Reverse partially bleached. On gallic and tannic acid agars, diffusion zones strong, no growth. On gentian-violet agar growth good, medium discoloured in 4 weeks. These prove that the fungus causes white rot.

(*b*) *Hyphal characters*. Advancing zone: hyphae hyaline (in the form of distinct rhizomorphs at the periphery), thin-walled and with many cross-walls, branched, without clamp connexions (Text-fig. 1*f*), 2·9–7·3 μ broad, sometimes hypha ends in a short swelling (Text-fig. 1*g*). Aerial mycelium: (*a*) hyphae as in advancing zone; (*b*) hyphae hyaline, thick-walled, unbranched with a few simple septa (Text-fig. 1*h*), 2·9–5·8 μ broad; (*c*) cuticular cells present, irregular, forming a pseudo-parenchyma (Text- fig. 1*i*).

P. lignosus and *P. zonalis* were paired on a thin agar smear on cover-glasses. When the two fungi met, the preparation was fixed, stained in cotton blue and examined under the microscope. It was observed that though hyphal fusions within each of the two species were common, there was no hyphal fusion between *P. lignosus* and *P. zonalis*, hyphae of one moving freely into those of the other.

There exists a good deal of confusion about the identity of *Hevea* root-disease fungus known as *Fomes lignosus* (Klotzsch) Sacc. Petch (1928) cleared up some of these confusions; it was, first of all, wrongly named as *F. semitostus* Berkeley. Petch, when at Kew in 1910–11, corrected it as *F. lignosus*. Lloyd, also then at Kew, agreed to this identification but subsequently Lloyd (1915) confused the real *F. lignosus* with *P. zonalis* and *P. microporus*. Petch (1921) distinguished *F. lignosus* from *P. zonalis* by two principal characters, viz. (1) *F. lignosus* is usually a parasite while *P. zonalis* is a ubiquitous saprophyte, (2) *F. lignosus* when broken into two shows two colours, the white context and the sharply contrasted red brown pore tubes, while *P. zonalis* is uniformly white internally. But subsequently Petch (1928), while discussing fully the identity of *F. lignosus*, remarked that the distinction of colour, on which he had earlier stressed so much, completely broke down in some forms of *P. zonalis*. He therefore concluded that both *F. lignosus* and *P. zonalis* are synonyms and that the rubber root-disease fungus of the East (Ceylon and Malaya) is an unnamed species and that it cannot be either *F. lignosus* or *P. microporus*.

Moreover, Petch was under a little misapprehension that Klotzsch deposited his type in *Herbario regio Berolinensi* when he remarked: 'I am unable to visit Berlin and to check by inspection of Klotzsch's type specimen'. The type specimen of Klotzsch is at Uppsala, Sweden, where we had sent in 1955 our specimen of *P. lignosus* to Dr J. A. Nannfeldt. It was examined by Dr Seth Lundell through the courtesy of Dr Nannfeldt

and compared with the type of *P. lignosus*. After critical study Dr Lundell had no hesitation in referring our fungus to *P. lignosus*. He further draws attention to Bresadola's studies on type specimens, according to which *P. auberianus* Mont., *P. diffusus* Fr. and *P. kemphoveneri* Fr., are synonymous with *P. lignosus*. One of us (S. R. B.) has recently obtained typical specimens of perfect sporophores of *P. lignosus* from diseased rubber (*Hevea brasiliensis*) roots kindly sent by the Rubber Research Institute of Ceylon, from which the fungus is growing in artificial culture in the laboratory.

On a careful consideration of all the facts we therefore conclude that *P. lignosus* and *P. zonalis* are two distinct species, and are not synonyms as finally held by Petch (1928). The two species are distinguished by the following five characters: viz. (1) *P. lignosus* is usually a parasite while *P. zonalis* is commonly a saprophyte; (2) the presence of encrusted cystidia in *P. zonalis* noted by Bose (1938) distinguishes it from *P. lignosus* where they are absent; (3) hyaline living hyphae are thin-walled and septate in *P. lignosus* while they are thick-walled and mostly non-septate in *P. zonalis*; (4) the two fungi are distinguished by their cultural characters where rhizomorphic strands develop in *P. lignosus* in the same way as they develop in nature. Such strands are absent in *P. zonalis* (Pl. 4, fig. 4), both in nature and in culture; (5) absence of hyphal fusions between *P. lignosus* and *P. zonalis* when the two fungi are paired. The fungus occurs usually in *Polyporus* form and rarely in *Fomes* form for which it was also named as *F. lignosus*. The present collections are all with only one pore-tube layer and are thus referred to as *P. lignosus*.

Incidentally, it may be remarked that when Overholts (1953) recorded *P. zonalis* as causing widespread and serious heart rot of various living trees in Louisiana in 1931, we suspect he was probably dealing with *P. lignosus*.

Finally, it should be noted that this is one of the many examples where cultural characters may be of great help in clearing up the confusion between two related species when morphological and anatomical characters do not help much.

Our sincere thanks are due to Dr J. A. Nannfeldt and Dr Seth Lundell for their valued opinion. The senior author (S. R. B.) thanks the West Bengal Government, Department of Education, for help with a research grant for this work.

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EXPLANATION OF PLATE 19

Fig. 1. Sporophore of *Polyporus lignosus* at the base of a living *Bauhinia purpurea* tree.
Fig. 2. Rot due to *P. lignosus* in *B. purpurea*. ($\times \frac{5}{6}$.)
Fig. 3. Culture of *P. lignosus*, 10 days old.
Fig. 4. Culture of *P. zonalis*, 10 days old.

(*Accepted for publication* 26 *January* 1957)

Pseudoeurotium indicum (Chattopadhyay & Das Gupta) Comb.Nov.

Since the description of *Thielavia indica* by Chattopadhyay & Das Gupta (these *Transactions* **40** (2), pp. 277–278, 1957), my attention has been drawn by Dr A. H. S. Brown of the Commonwealth Mycological Institute, Kew, to the publication of a paper by A. C. Stolk, 'The genus *Anixiopsis* Hansen and *Pseudoeurotium* van Beyma'. As the ascospores show no perceptible germ pore, *Thielavia indica* is better placed under *Pseudoeurotium*, but as it does not match any other material or description of the known species of *Pseudoeurotium* van Beyma *Thielavia indica* Chattopadhyay & Das Gupta (loc. cit.) is, therefore, renamed **Pseudoeurotium indicum** (Chattopadhyay & Das Gupta) comb.nov.

S. B. Chattopadhyay, *State Agricultural Research Institute, Calcutta, India*

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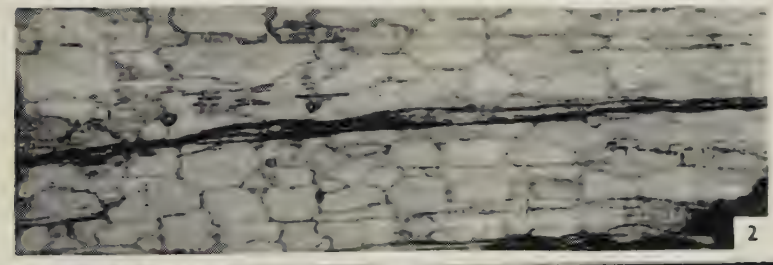

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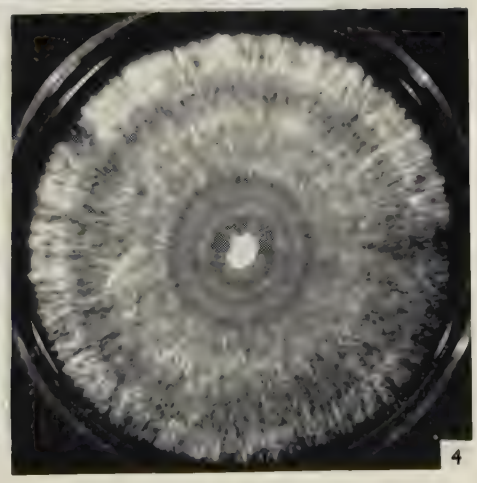

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Trans. Brit. mycol. Soc. **40** (4), 461–471 (1957).

PIPTOCEPHALIS VIRGINIANA SP.NOV.

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(With 4 Text-figures)

The morphology and zygospores of a new homothallic species of *Piptocephalis* are described. A discussion involves its comparison with other species, and a consideration of some general points of taxonomy.

Piptocephalis virginiana sp.nov. (Figs. 1–4)

Sporophori erecti, circa 6 mm. alti et plerumque in 6–8 dichotomias distales divisi, nec rhizoides nec stolones habent. Sporophori juventute 'Cartridge-buff', maturitate 'Sweetmeat', dichotomiis distalibus exceptis distincte striati. Cellae capitales obconicae, superne semi-globosae, latitudine maiores quam altitudine, apice paulo planatae, basi incrassatae, in tuberculis apice incrassatis 11–30 catenas sporarum ferunt, quarum quaeque 3–8 sporas cylindratas habet. Cella capitalis (3·5 μ)–5·5 (8) μ lata, sporae 3–9 μ longae et 1·5–2·2 μ latae (5 × 2 μ). Zygosporae (17·5)22·5–30(38) μ latae, gemmis circum heterogametangiorum juncturas dispositis formatae. In exposorio putei parvuli, parietibus regularibus vel irregularibus, pentangulis vel sexangulis. Monoecia.

Sporophores erect, averaging 6 mm. in height, without rhizoids and stolons, and generally branching distally into 6–8 dichotomies. Sporophores Cartridge Buff (Maerz & Paul, 1950) when young, Sweetmeat at maturity, possessing striations apparent except on terminal dichotomies. Obconico-spherical head cells, greater in width than height, slightly flattened apically with thickened bases, and bearing on apically thickened tubercles 11–30 spore chains with 3–8 cylindrical spores per chain. Head cell with a diameter range of 3·5–8 μ (mean 5·5 μ) and spores 3–9 μ long and 1·5–2·2 μ wide (mean 5 × 2 μ). Zygospores (17·5) 22·5–30 (38) μ diam., formed by delimitation of buds formed around the point of fusion of heterogametangia. Exospore pits with regular or irregular pentagonal or hexagonal walls. Homothallic.

This species was found on *Mucor hiemalis* in material collected from a cave near Morgantown, West Virginia, U.S.A.

Methods

All cultures were grown on potato-dextrose agar at 26° C., and all measurements made from water-mounts. Material for *in vivo* studies was prepared by a modification of Vernon's (1931) technique. Under sterile conditions, a supply of almost setting medium is suitably inoculated and a drop of appropriate volume is pressed on a slide with a circular coverslip, so that a medium of adequate depth is pressed to within 3 mm. of the coverslip edge. Incubation of such slides in moist chambers permits the inspection, at prescribed times, of all stages from germination to sporophore and zygospore formation.

Morphology

The morphological interpretation and terminology of parts in *Piptocephalis* fructifications differ from author to author. To avoid implied speculation, Dobbs & English (1954) use purely descriptive terms. Because of the merit of this, their terms spore rod, head cell, spore chain are used here.

Sporophores are formed in 48 hr. and darken to Sweetmeat when 7 or more days old. The transition from the sporophore to the mycelium is often marked by a sudden attenuation. The principal axis has an average diameter of 6 μ and the dichotomies occupy only the uppermost third of the sporophore. Distal dichotomies are collected into groups so that each has four terminal dichotomies occupying, in plan, the corners of a rectangle. Gradual attenuation leaves the branches of the terminal dichotomy 2 μ wide and 10–30 μ long.

Septation proceeds from the base towards the sporophore tips and septa develop centripetally. These, in the principal axis, may be infrequent or closely set at 10 μ intervals. In the dichotomous portion, septa are formed in pairs slightly above or at the points of bifurcation. It is rare, however, to find the terminal dichotomy so delimited. Intense activity of dense protoplasm initially characterizes the developing sporophore. Later, this dense protoplasm progresses with the sporophore leaving basal portions, which are successively septated, with a clear fluid and attenuate protoplasm. This apparent suppression of protoplasmic activity, with time, towards the distal part of the sporophore is quite striking, especially as the older septated portions appear to become quite empty.

With all dichotomies established, their tips swell to become spherical after passing through blunt dichotomous stages. Each swelling, when 3–4 μ diam., develops projections which are, in turn, spherical, then oval and finally elongate into cylindrical spore rods (Fig. 1 D). These are constricted near their points of origin and attain an average length of 25 μ. Meanwhile, the swelling has achieved its maximum diameter and becomes slightly flattened apically.

Two processes, more or less simultaneous, now delimit each swelling as a head cell. The first, spore formation, is preceded by the appearance of clear hyaline zones of protoplasm in the spore rod (Fig. 1 D). On either side of each zone, walls are laid down which link up with the spore-rod membrane so that the hyaline zone is now interstitial. The walls increase in thickness, becoming compound and encroaching on the hyaline protoplasm. Each loculus, so formed, secretes a uniform wall closely adherent to the locular wall to become a spore. The basal hyaline zone arises in the constriction of the spore rod already noted. On the distal side of the zone normal spore production proceeds whilst a prominent deposition of thickening is laid down on the proximal side. Thus during spore chain formation a portion of the constriction is left as a tubercle with apical thickening on the upper surface of the terminal swelling. These tubercles are approximately 1 μ diam. and 0·5 μ long.

The second process is the septation which occurs in the confluence of the swelling and the branch bearing it. Here, too, septa are laid down on either side of a hyaline zone of protoplasm so that it becomes interstitial

(Fig. 1 D). On each septum a further heavy and characteristic thickening is deposited. This leaves the now delimited head cell with a uniform pad of basal thickening and the sporophore tip with a thickening shaped like an inverted tumbler (Fig. 1 A, D).

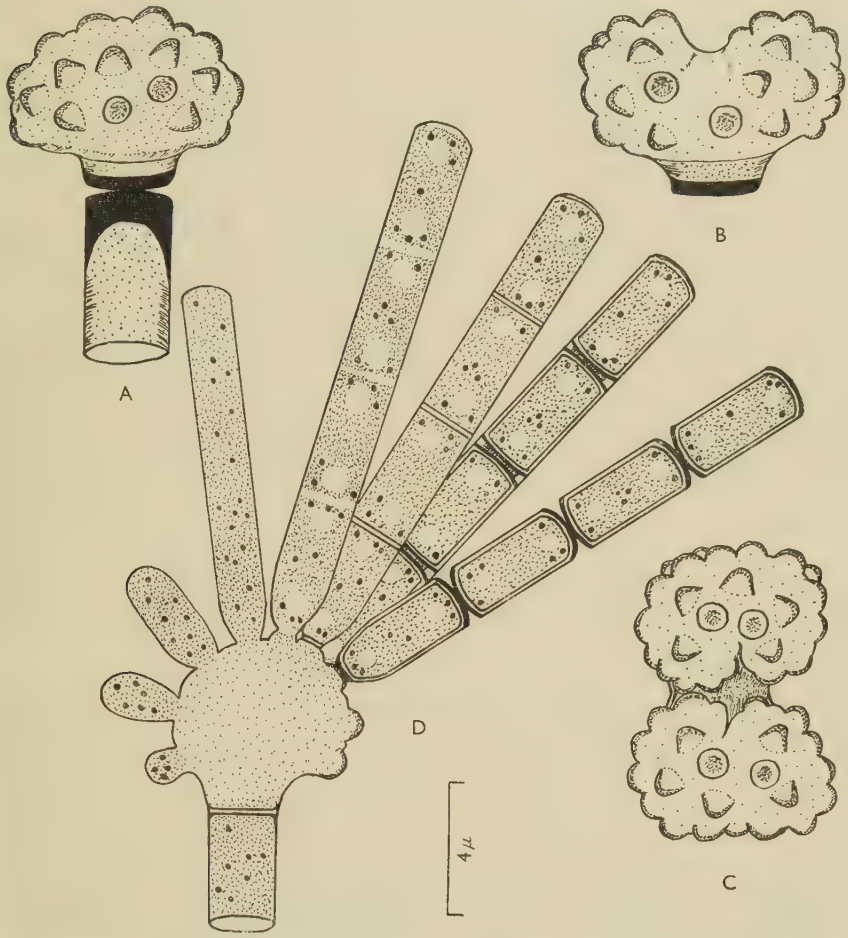

Fig. 1. *P. virginiana*. A, head cell and sporophore tip; B, dichotomous pleomorphic form of the head cell; C, dichotomous pleomorph viewed from above; D, composite diagram showing the ontogeny of spore chains and delimitation of the head cell.

The form of the normal head cell is featured in Fig. 1 A. Pleomorphic forms have approximately a 1 % incidence and they exhibit varying degrees of lobing. A dichotomous form is shown in Fig. 1 B, C, but extreme forms may exhibit sixteen or more lobes.

The disjunction of the spore rods into spores and the separation of the head cell from the sporophore occur in similar fashion, but the latter has not been so fully observed as the former. In the spore rods, centripetal disorganization of interstitial zones of hyaline protoplasm begins. This is

not preceded by the dissolution of the original spore-rod membrane. The latter can still be seen when the interstitial layers have disorganized to short, median, cylindrical plugs. Accompanying the breakdown of the interstitial layer is an outward curvature of the end-walls of the future spores. Although not strictly observed it is felt that this is responsible for the fracture of the spore-rod membrane at points opposite to the interstitial layers. The separation of the spores is finally achieved by the full dissolution of interstitial protoplasm.

The above details of spore formation and disjunction are given because they do not fully agree with existing accounts of which Mangin's (1899) description for *P. arrhiza* Van Tieghem & Le Monnier (1873) is probably the best.

The cylindrical spores with their rounded ends and smooth walls characteristically possess two terminal vacuoles around which most of the spore nuclei are situated. These vacuoles pre-exist in the spore rod prior to septation.

Separate counts of 100 on each of thirteen different hosts have been used to establish the size-range and mean sizes for spores and head cells, spore numbers per spore rod, and spore rods per head cell. A comparison of the data obtained from these hosts showed good uniformity, although *Mucor fragilis* tended to produce large parasite spores. All counts and measurements were taken on 7-day-old cultures and the hosts concerned

Table 1. *Hosts of* Piptocephalis virginiana

Mucor fragilis Bain.
Circinella (?) *tenella* (Ling–Young) Zycha
Mycotypha microspora Fenner
Mucor hiemalis Wehm. (+)
M. hiemalis Wehm. (−)
Rhizopus stolonifer (Ehrenb. ex Fr.) Lind.
Cunninghamella elegans Lendner
Thamnidium elegans Link
Actinomucor repens Schost.
Syncephalastrum cinereum Bain.
Pilaira anomala (Ces.) Schroet.
Absidia blakesleeana Lendner
Phycomyces nitens (Agardh) Kunze ex Fr.
Helicostylum pyriforme Bain.
Mortierella isabellina Oud.
Zygorrhynchus moelleri Vuill.

Table 2. *List of fungi not parasitized by* Piptocephalis virginiana

Pythium aphanidermatum (Edson) Fitzp.
Allomyces cystogenes Emerson
Mortierella stylospora Dixon-Stewart
M. hygrophila Linnem. var. *minuta* Linnem.
Conidiobolus villosus Martin
Penicillium chrysogenum Thom
P. waksmani Zal.
P. citrinum Thom
P. claviforme Bain.
P. camemberti Thom
P. roqueforti Thom
P. frequentans Westl.
Aspergillus parasiticus Speare
A. flavus Link ex Fr. var. *rufus* Blochw.
A. repens (Corda) Sacc.
A. niger v. Tiegh.
Saccharomyces cerevisiae Meyen ex Hansen
Neurospora tetrasperma Shear & Dodge
Streptomyces griseus (Krainsky) Waksm. & Henrici
S. fradiae (Waksm. & Curtis) Waksm.

are the first thirteen listed in Table 1. Of the fungi tested, Table 1 lists those which acted as hosts, and Table 2 those which allowed no parasitization. It will be noted that all hosts are mucorine and that *P. virginiana* does not extend its host range to the Ascomycetes favoured by *P. xenophila* Dobbs & English.

Germination

Spores swell to become almost spherical except for terminal caps which represent the end-walls. The vacuoles merge to form a large central vacuole and nuclei multiply. Germ tubes, approximately 0·8 μ diam., are short and simple if quick contact is made with a host. Otherwise they become quite long, bearing short branches set at right angles to themselves and their main branches. Contact with a host made by a tip of the branch system results in a small oval appressorium and the host is penetrated by a minute tube which enlarges distally to form a simple, or rarely, a bilobed primary haustorium (Fig. 2 G, H).

During latter stages of germination the spore is occupied by a large vacuole and resident protoplasm is only obvious at the exit points of germ tubes (Fig. 2F). Once host contact is established, there commences a continuous transfer of protoplasm back to the spore and it quickly refills. As a consequence the parent spore and all its germ tubes increase in diameter whilst the appressorium elongates. Further mycelial extension now continues from either an existing germ tube which has or has not yet contacted the host or directly from the spore (Fig. 2F–H). The host-sustained secondary mycelium is 1·5–3 μ thick and is characterized by appressoria and haustoria quite different from the primary type. The appressoria may be terminal, lateral and sessile on any branch or may arise from the angle of a dichotomy, one or both of whose branches may develop for a few microns only (Fig. 3B).

No stoloniferous habit exists here. The appressorium, approx. 8 μ wide, is typically obcordate but form variation usually involves a suppression of lobing. The penetration tube arises between the lobes and distally expands into two to five lobes, one to five of which may be dichotomously branched (Fig. 3A–D).

The parasite mycelium is initially non-septate and shows an intense activity. This activity moves to points where new contacts are made with the host and the older mycelium loses its content and becomes septate.

Dwarf Sporophores

In the absence of a host and on suitable media the spores of *P. virginiana* produce one generation of spores on dwarf sporophores. These spores, although unable to germinate in the continued absence of a host, are viable and will parasitize a suitable host. This statement applies to all species and isolates tested except *P. xenophila* Dobbs & English. The spores of this somewhat specialized species will germinate on certain media but will not produce dwarf sporophores.

In *P. virginiana* germ tubes, which develop upwards, give rise at 40 hr. to sporophores 0·2 mm. high. These latter are straight and unbranched or possess one dichotomy. Occasionally three branches may be seen where one branch undergoes a second dichotomy. Branch tips acquire swellings, 3 μ diam., which are slightly flattened and thickened apically and sometimes 2 to 3-lobed. These swellings eventually bear four to seven spore chains, of two to three spores, borne on minute tubercles. The average

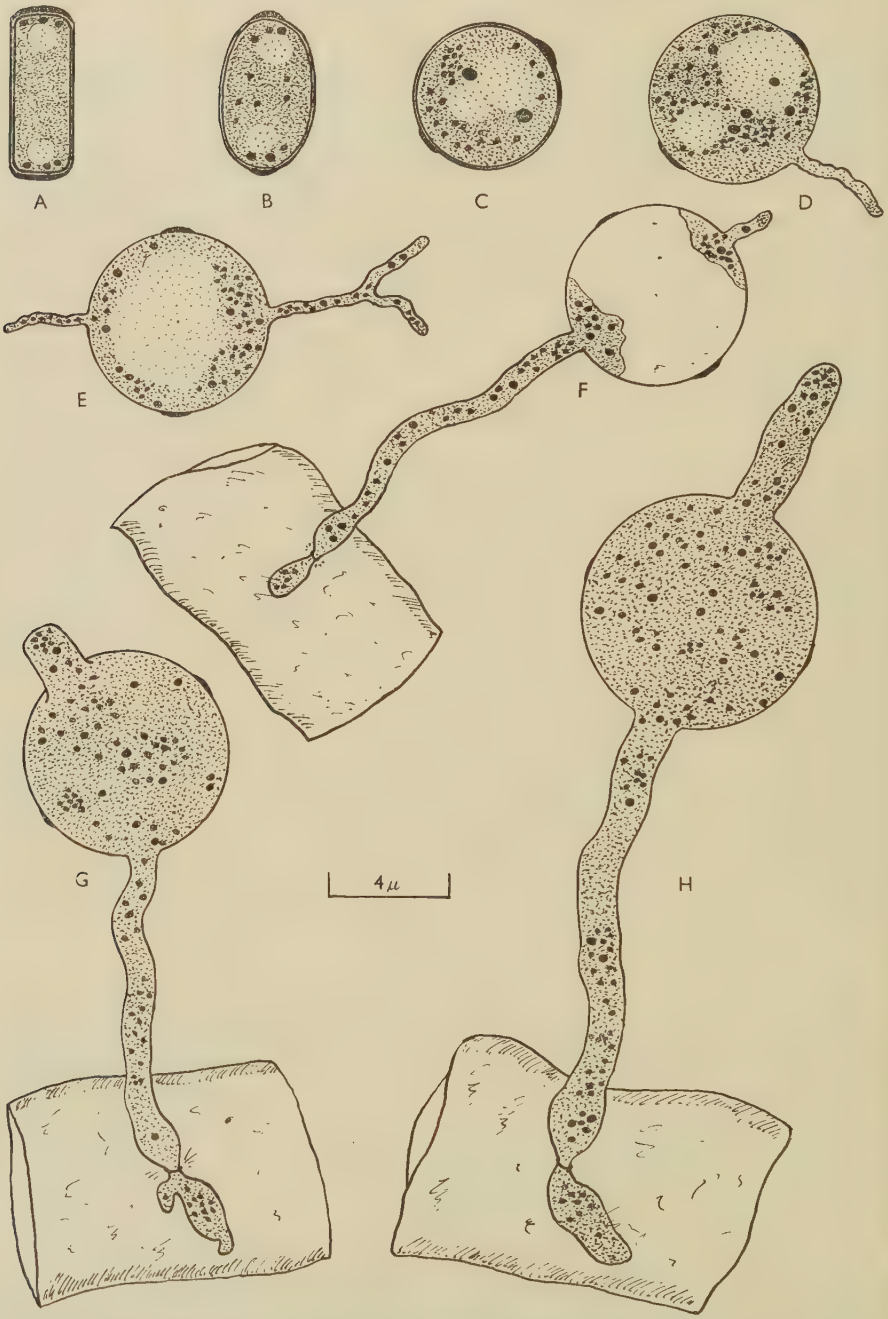

Fig. 2. *P. virginiana*. A–E, early stages in spore germination showing swelling, terminal caps, increase in number of nuclei, vacuolar fusion, and germ tubes; F, spore with some resident protoplasm and a germ tube with a simple primary haustorium; G, germ tube with bilobed primary haustorium; G, H, spores refilled with protoplasm and commencing to send out the secondary mycelium.

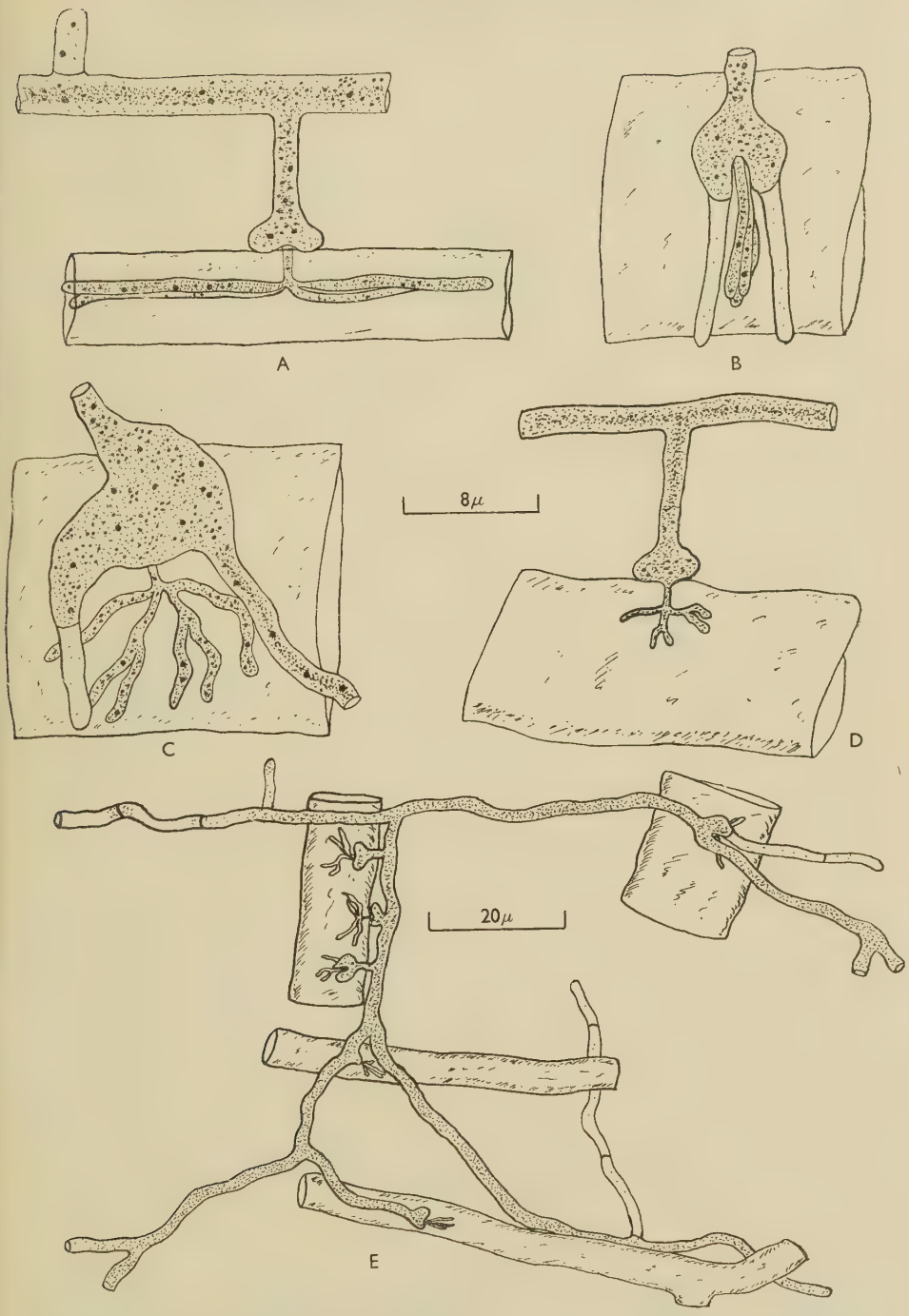

Fig. 3. *P. virginiana*. A–D, secondary appressoria and haustoria; E, general habit of mycelium. A, terminal on short lateral branch; B, abortive continuations of the appressorium; C, appressorium with two continuations, one being abortive; D, frequent non-obcordate type of appressorium.

spore dimensions are $5{\cdot}5 \times 2{\cdot}2\,\mu$ and no head cell is delimited. Agar or surface germ tubes branch, are septate, never give rise to sporophores and achieve lengths of up to 1 mm.

The individual development of spores indicated is quite surprising, and one is led to consider what is possessed by a spore that enables it to develop to a certain extent only, and what type of contribution is made by the medium.

Zygospores

Mature zygospores are spherical or subspherical and light orange-brown in colour, with walls $1{\cdot}5$–$2{\cdot}2\,\mu$ thick. The colour resides in the stratified exospore which exhibits a wall architecture almost identical with that in *P. cylindrospora* Bain. The exospore is, however, only partially penetrated by pits and there is, therefore, no delicate replica of the exospore architecture on the surface of the endospore. Local coralloid portions on the exospore occur by partial breakdown of the walls of adjacent pits.

The exospore extensions are smooth and proceed with sudden attenuations as far as the septa delimiting gametangia and suspensors. The three-layered endospore has a thick stratified middle layer and the zygospore contents show green globules. The zygospore limbs are not widely separated and the suspensor components of these often show extra septation and hypertrophy.

Development. This follows the pattern already described for *P. cylindrospora* Bain, and *P. xenophila* Dobbs & English by Leadbeater & Mercer (1956, 1957). Apposed, cylindrical, copulation branches with simple, basal coiling develop and differentiate into gametangia and suspensors. The copulation branches continue to elongate and enlarge, maintaining a cylindrical to clavate form (Fig. 4A). The marked budding following fusion results in a separation of the gametangial limbs, which have hitherto remained in close apposition (Fig. 4A–C). The deposition of the exospore is especially heavy at points below the confluence of the bud and the future gametangial remnants. These points mark the position of the septa which eventually delimit the zygospore. Because of the position of these septa, the endospore has two noticeable basal projections (Fig. 4F). The residual cytoplasm in a gametangial remnant is quickly consumed to form a thin, complete, internal film of appositional thickening (Fig. 4F). The zygospores are produced in the agar directly from the normal mycelium and single spore isolates show this species to be homothallic. Within a temperature range of 10–30° C. zygospores are readily produced on a variety of media and all mucorine hosts recorded in the text. Double and single azygospores are frequently formed locally (Fig. 4G–H).

Development studies on numerous individual zygospores show that full zygospore production takes from 48 to 72 hr.

Discussion

The obconico-spherical type of head cell as described for *P. virginiana* is featured by several isolates in our possession. In previously described species only *P. lemonnierana* Vuillemin (1902) has a description which

truly fits this type of head cell. However, outstanding differences are shown by the latter with respect to all dimensions, habit, spore numbers, spore shape and formation, etc.*

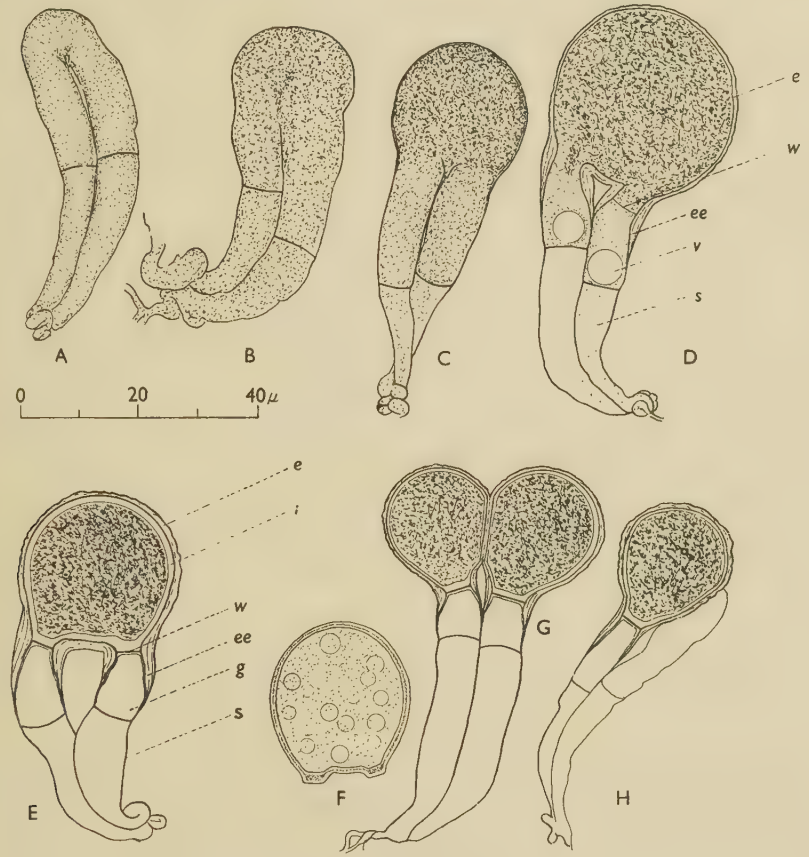

Fig. 4. *P. virginiana.* A–E, zygospore formation. A, fusion of gametangia; B, early budding; C, bud with exospore and protoplasmic differentiation; D, commencement of zygospore delimitation; E, mature zygospore; F, zygospore with exospore removed, showing two prominent basal projections of the endospore; G, double azygospore. H, single azygospore. *e*, exospore; *ee*, exospore extension; *g*, gametangial remnant; *i*, endospore; *s*, suspensor; *v*, vacuole; *w*, delimiting septum.

It is apparent that the morphology of the head cell in *Piptocephalis* is secondarily derived. In all species and isolates examined a blunt dichotomy becomes a terminal spherical enlargement. Its subsequent form depends on: (*a*) the position of the delimiting septum in the swelling and the branch bearing it; (*b*) the degree of attenuation at the point of septation; (*c*) apical changes, sometimes absent, accompanying spore-rod formation and septation which result in a range involving a mere flattening to a marked

* *P. lemonnierana* possesses interesting features, and one wonders why it is made a synonym of *P. freseniana* by Zycha (1935). An isolate, here, resembles *P. lemonnierana* in several features and it is felt that the integrity of the latter is warranted.

dichotomous lobing; (*d*) the position of septa, delimiting spore chains from the head cell, which determine the presence and prominence of tubercles.

It is apparent, thus, that perfectly spherical head cells cannot occur in the genus and it is felt that several of the erected species have had their head cells too readily described as spherical. For this reason all such species have been compared with *P. virginiana* and all, except *P. sphaerocephala* Mangin (1899), eliminate themselves by possessing some outstanding feature. The latter species was provisionally erected from studies made on pickled material only and the description is completely inadequate for comparative purposes. As points of difference, it possesses (*a*) short, heavily striated terminal dichotomies which, initially broad, become markedly attenuate; (*b*) a scattered distribution of tubercles on the head cell; (*c*) spores 3·3 μ long by 1·5 μ wide. Except for Mangin's other doubtful species *P. monospora*, 1899, the spore dimensions are the smallest yet recorded in any erected species. The position of *P. sphaerocephala* is treated differently by Naumov (1939) and Zycha (1935). The former permits it to stand as an independent species. Zycha makes it a synonym of *P. freseniana* De Bary (1865), a move which is not justified, and which can only be a convenient way of disposing of this ill-described and doubtful species.

The closest relative to *P. virginiana* is an isolate, available at Baarn, deposited by Claussen and incorrectly diagnosed as *P. freseniana* De Bary. This has been under study for 3 years and has maintained its features but never produced zygospores although subjected to a variety of experimental conditions. Close parallel study of the two brings out small cumulative differences which enable them to be distinguished. The differences which exist cannot fully justify their separation, and a suggestion that the Baarn isolate might be an azygosporic form of *P. virginiana* could not meet strong resistance.

The zygospores of *P. virginiana* confirm a general pattern of formation in the genus but the method of formation described for *P. freseniana* De Bary by Brefeld (1873) and Bainier (1907) must be unusual or suspect. It has been previously stated by the authors (1957) that the wall architecture of the zygospores could be used as a strong additional criterion in the taxonomy of the genus. This statement was based on the fact that the zygospores of *P. freseniana* De Bary, *P. cylindrospora* Bain., and *P. xenophila* Dobbs & English, all possessed diagnostic wall architecture. Since then, the type of wall architecture noted in *P. cylindrospora* Bain. has been seen in *P. virginiana* and three different isolates under investigation, yet the value of zygosporic features is not impaired since all these zygospores, with similar wall architecture, still display other differences of taxonomic merit.

Cultures have been deposited at the Commonwealth Mycological Institute, Kew, and the Centraalbureau voor Schimmelcultures, Baarn.

The authors wish to thank Prof. H. L. Barnett, West Virginia University, for providing material for this study and Mr J. B. Cooper, Research Library, Boots Pure Drug Co. Ltd., for the Latin diagnosis.

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(*Accepted for publication* 3 *December* 1956)

ADDENDUM

A recent paper by Berry & Barnett (*Mycologia*, **49**, 374–386, 1957) discusses primarily the physiology and host range of *P. virginiana* but also includes a brief survey of its morphology. Various dimensions which they quote differ somewhat from those in the above text. It is surprising to find the spore width quoted as 2·6 μ when this dimension has never been achieved as an upper limit in our cultures. However, all the differences may be related to the culture techniques adopted and the use by Berry & Barnett of *Choanephora cucurbitarum* as a standard host.

Other overlapping material in both papers agrees well, but it is noted that of the nine *Mortierella* species tested by Berry & Barnett only *M. pusilla* was parasitized. During the course of our work, and in conjunction with Mrs M. Turner, Department of Botany, University of Nottingham, nine species of *Mortierella* were also tested as hosts for *P. virginiana*, *P. xenophila* Dobbs & English and one other *Piptocephalis* isolate. In all cases *M. ramanniana* (Möller) Linnem., *M. isabellina* Oud. & Köning, *M. vinacea* Dixon-Stewart, and *M. longicollis* Dixon-Stewart were parasitized. Except for the last species, it cannot be claimed that the host range within *Mortierella* has been extended because of questions of synonymy that arise. But the results may have some bearing on the latter problem and the possession of mucorine characters by some *Mortierella* species. The following species refused to act as hosts: *M. humilis* Linnem., *M. globulifera* Rostrup, *M. bainieri* Cost., *M. alpina* Peyronel, *M. polycephala* Coem.

Trans. Brit. mycol. Soc. **40** (4), 472–476 (1957).

THE CLAVARIACEAE OF THE MUSSOORIE HILLS (INDIA). VII

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(With Plate 20 and 3 Text-figures)

Three new species are described: *Pterula decumbens*, *Ramaria echino-virens*, and *Typhula longispora*. Type collections are in the herbarium of the Panjab University: duplicate material in alcohol formalin is at the Botany School, Cambridge.

This paper records three new species of clavarias from the Mussoorie Hills (5000–7500 ft. altitude in the north-west Himalayas), as a part of the study of the fungal flora of the region undertaken by Dr Thind and his students (see References). The first six contributions described 32 known clavarias (22 species, 5 varieties, 5 forms) and 11 new ones (5 species, 6 varieties). The fruit-bodies have been described from living material, supplemented with dried material and that preserved in alcohol formalin. The classification of Corner (1950) has been followed. The numbers of the species are the serial numbers of this Clavarioid Flora.

44. **Pterula decumbens** sp.nov. (Pl. 20; Text-fig. 1)

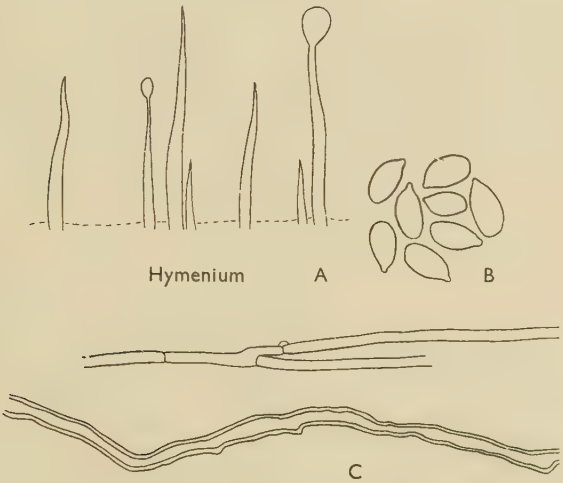
Receptacula ramosa, decumbentia, repentia vel suberecta, dense intermixta, pallide brunnea, apicibus albis, trunco nullo, hinc inde rhizoideis tenellis affixa; axi primo cylindrico, ad 3 cm. long, ad 0·33 mm. lato, ramis divaricatis, angustioribus, prostratis, numerosis, adventitiis praedito. Sporae 4–7·2 × 2·5–4 μ, hyalinae, laeves, ellipsoideae. Sterigmata 4. Cystidiola 32–36 × 2·5–4 μ, hyalina, lanceolata, acuta vel subcapitata, tenuiter tunicata, numerosa, ad 26 μ projicientia. Hyphae biformes, skeletales crasse tunicatae et brunneolae.

Fructifications *branched*, *decumbent* or suberect, *densely intermixed* and covering the substratum for several centimetres, slender, *light brown*, paler to the white tips, here and there attached by numerous fine rhizoids; trunk absent; main axis –3 cm. × 0·33 mm., cylindric; branches adventitious, numerous, –0·26 mm. wide, singly or clustered, prostrate, the sterile tips acute to acuminate, varying obtuse; flesh white; taste and smell inparticular.

On dead twigs, leaves and needles in Cedrus-forest: India (Kadu Khal, Mussoorie, 3 September 1955, No. 120, *typus*).

Hymenium amphigenous, absent from the rhizoids and parts of the main axis in contact with the substratum, thickening to 80 μ. *Cystidioles*

32–36 × 2·5–4 μ, lanceolate, hyaline, thin-walled, acute or somewhat capitate, projecting 10–26 μ. *Basidia* 18–24 × 4–6 μ, clavate, clamped; sterigmata (2–)4, straight, 2·5–5 μ long. *Spores* 4–7·2 × 2·5–4 μ, hyaline, ellipsoid, smooth, aguttate, the apiculus prominent, –1 μ long. *Hyphae* dimitic; generative hyphae 3–5 μ, hyaline, thin-walled, clamped; skeletal hyphae 2–6·5 μ, light brown, the walls –1 μ thick, sometimes branched, often undulate, rarely septate (without clamps).



Text-fig. 1. *Pterula decumbens* Corner, Thind & Dev. A, cystidioles, × 1150; B, basidiospores, × 1150; C, hyphae, skeletal and generative, × 1000.

This fungus has adventitiously branched fruit-bodies as in many species of *Pterula*, but, instead of being erect or horizontally projecting, they are decumbent and attached in places by fine rhizoids. This habit suggests the step towards the condition in *Allantula* Corner, the fruit-body of which consists practically of fertile rhizoids. It is intended to study more fully the development of *P. decumbens* in the attempt to elucidate its form-factors.

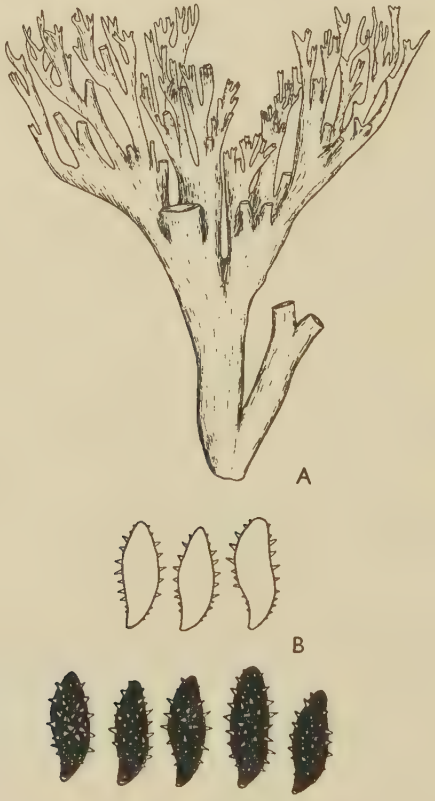
45. **Ramaria echino-virens** sp.nov. (Text-fig. 2)

Receptacula ad 13 × 7·5 cm., multiramosa, rugulosa, brunneo-luteola, superne aurantio-lutea, basi albo, trunco nullo; ramis inferne polychotomis, ad 15 mm. latis, superne dichotomis, saepe inaequalibus, apicibus obtusis; carne luteo-viridi, incisa vel contusa intensius virescenti. Sporae 9·5–13 × 2·8–4·8 μ, brunneae, echinulatae, ellipsoideae, echinulis –0·8 μ longis. Sterigmata 2–4. Hyphae ad 12 μ latae, fibulatae, tenuiter tunicatae.

Fructifications 7·5–13 × 4·5–7·5 cm., gregarious, erect, *massive, profusely branched from the base*, trunk absent, rugulose, *light brownish yellow below, orange yellow above*, base white; branches cylindric, polychotomous and –15 mm. wide below, dichotomous above in alternating planes, often unequal, ultimate branchlets small, –5 mm. long, the blunt fertile apices orange yellow; *flesh soft, greenish yellow, turning deep green on cutting or bruising*; smell inparticular; taste starchy.

On soil under oak-forest: India (Sarkunda road, Mussoorie, 12 Sept. 1954, No. 121, *typus*).

Hymenium amphigenous, thickening to 180 μ. *Basidia* 7–8·5 μ broad; sterigmata 2–4, massive, straight, 3·5–7 μ long. *Spores* 9·5–13 × 2·8–4·8 μ (excluding the spines), with dark brown, slightly thickened wall, narrowly ellipsoid or navicular, straight or slightly curved, *echinulate* with spines



Text-fig. 2. *Ramaria echino-virens* Corner, Thind & Dev. A, fruit-body with blunt apices, × ½; B, echinulate spores, × 1150.

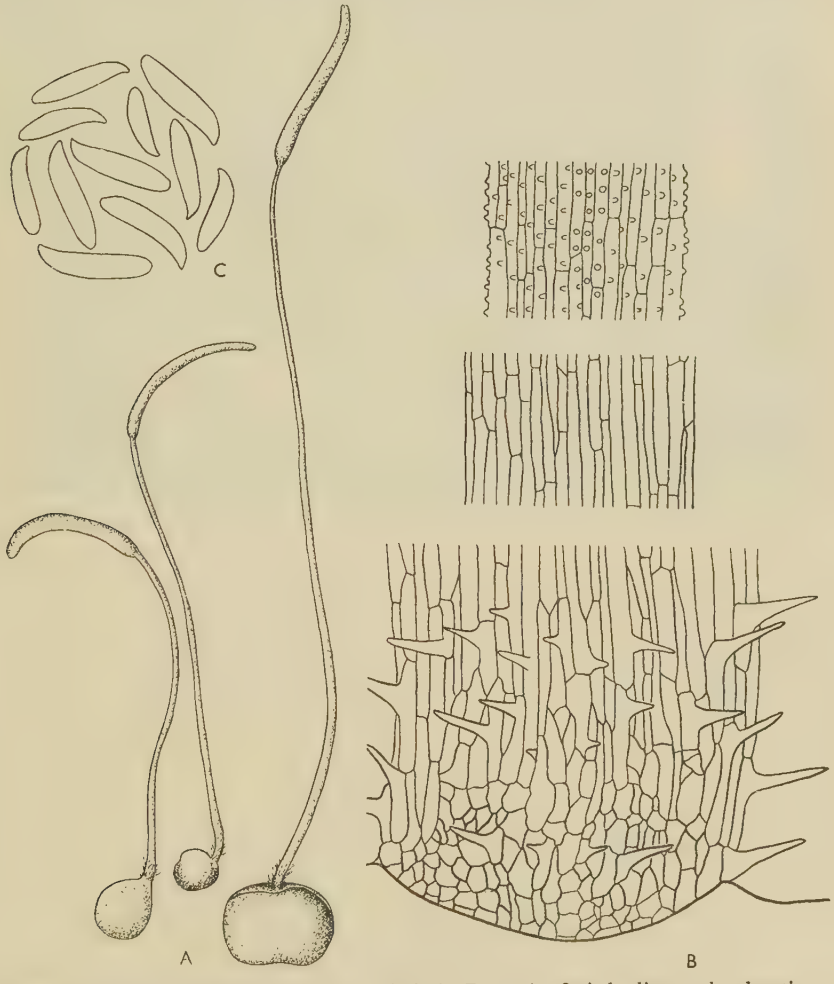
–0·8 μ long, the spines most conspicuous and abundant in the broader part of the spore, shorter and sparser towards both ends, aguttate, apiculate. *Hyphae* monomitic, 2–12 μ wide, cells –270 μ long, hyaline, thin-walled, branched, inflated, clamped, the septa at long intervals, often swollen and saccate on one side of the septum.

This new species is closely allied with *R. ochraceo-virens* (Jungh.) Donk, but is distinguished by the much larger fruit-body and the longer, more strongly echinulate, spores. Also, *R. ochraceo-virens* grows only under conifers.

46. **Typhula longispora** sp.nov. (Text-fig. 3)

Receptacula ad 25 mm. alta, tenuia, per sclerotium solitaria: capitulo 4–10 × 0·3–0·5 mm., albido, cylindrico, obtuso, carnoso, apice fertili: stipite 10–20 × 0·1–0·3 mm., apici 90–190 μ lato, filiformi, inferne brunneo, basi saturatiori et puberulo, superne albido pruinoso. Sporae 8–12 × 2–3 μ, subcylindricae. Sterigmata 4. Hyphae 3·5–10 μ latae, fibulatae, tenuiter tunicatae. Sclerotia 1–2·5 × 0·5–1·2 mm., globosa, brunnea, dein compressa et saturatiora, laevia; cellulis externis unistratosis, parietibus sinuosis brunneis 1–2·5 μ crassis, cuticula brunnea 4–8 μ crassa; cortice 20–50 μ crasso, medulla vix diverso, hyphis 4–10 μ latis contiguis, haud agglutinatis, contextis; medulla albida, hyphis laxe contextis, tunicis 1–2 μ crassis, raro tenuibus.

Fructifications to 25 mm. tall, one per sclerotium, erect, slender, simple, fleshy, brittle, smooth; *head* 4–10 × 0·3–0·5 mm., *white cylindric*, straight or curved, apex obtuse and fertile; *stem* 10–20 × 0·1–0·3 mm.,



Text-fig. 3. *Typhula longispora* Corner, Thind & Dev. A, fruit-bodies and sclerotia, × 5; B, parts of the stem, × 300; C, basidiospores, × 1150.

white, brown below, the base darker and puberulous, apex pruinose, filiform, 90–190 μ wide at the top, the middle part (7–13 mm. long) glabrous; smell and taste inparticular.

On fern leaflets and rachids: India (Depot Pound, Mussoorie, 14 Aug. 1955, No. 122, *typus*).

Hymenium –32 μ thick, not thickening. *Basidia* 26–29 × 3·5–4·5 μ, clavate; sterigmata 4, straight, 4–8 μ long. *Spores* 8–12 × 2–3 μ, hyaline, elongate ellipsoid to subcylindric, smooth, aguttate, apiculate. *Caulocystidia* at the base of the stem 8–44 × 2–6 μ, conical with brownish walls thickened up to 0·5 μ; at the apex of the stem as very short subcylindric protuberances –3 × 2 μ or less. *Hyphae* monomitic, 3·5–10 μ wide, the cells to 305 μ long, hyaline, thin-walled, branched, somewhat inflated, clamped, septate at short to long intervals, somewhat agglutinated at the surface of the stem.

Sclerotium 1–2·5 × 0·5–1·2 mm., spherical and brown, then compressed and darker, smooth; epidermal cells in one layer, irregularly oblong, *c.* 10–70 × 6–24 μ, the brown, undulate walls 1–2·5 μ thick, often with long or short rod-like projections into the lumen; cuticle 4–8 μ thick, brown; cortex 20–50 μ thick, not sharply delimited from the medulla, composed of contiguous, but not agglutinated, hyphae 4–10 μ wide; medulla white, composed of loosely interwoven hyphae 3–10 μ wide with walls 1–2 μ thick, a very few with thin walls and 2–6 μ wide.

This species does not seem nearly related to others which have been collected on fern remains, but its affinity is with *T. graminum* Karst., having the same sclerotial structure, but with longer and narrower spores and larger fruit-body. It is, possibly, *T. graminum* var. *filicina* Karst.

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EXPLANATION OF PLATE 20

Pterula decumbens Corner, Thind & Dev. × 3.

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(*Facing p.* 476)

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LEVEILLULA CLAVATA SP.NOV.

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(With 4 Text-figures)

Leveillula clavata sp.nov., which is described from *Euphorbia pulcherrima* Willd. from Nairobi, Kenya, differs from *L. taurica* (Lév.) Arn. mainly in the shape of the conidium.

Leveillula clavata sp.nov. (Figs. 1–4)

Mycelium partim superficiale et partim immersum, ex hyphis ramosis, septatis, hyalinis compositum; conidiophora ex apice lateribusque hypharum oriunda, erecta vel ascendentia, simplicia vel ramosa, recta vel flexuosa, hyalina, 38–40 μ longa, 6–9 μ crassa; conidia singula in apice conidiophori oriunda, clavata, hyalina, 38–76 × 10–16 μ; cleistothecia primo sphaerica et pallide brunnea, dein hemisphaerica vel pezizoidea et fusco-brunnea vel atra, 120–200 μ diam., 80–120 μ alta, appendicibus hyalinis ornata; asci 2-spori, 58–65 × 20–24 μ; ascosporae ovales, hyalinae, 18–26 × 12–14 μ; habitat in foliis vivis *Euphorbiae pulcherrimae*, Nairobi, Kenya, R. M. Nattrass, January 1950, Herb. I.M.I. 39647 (typus).

While investigating the host range of *Leveillula taurica* (Lév.) Arn. in the herbarium of the Commonwealth Mycological Institute, Kew, my attention was drawn by Dr M. B. Ellis to a specimen which differed from *L. taurica* in the shape of its conidia. The fungus was on *Euphorbia pulcherrima* Willd. collected in Nairobi by R. M. Nattrass in January 1950 (Herb. I.M.I. 39647).

The fungus appears on the lower (abaxial) side of the leaf where the superficial mycelium covers the surface irregularly. Cleistothecia were found abundantly on all the leaves, indicating that the disease was probably in a late stage of development. The pathological symptoms on the leaves of *E. pulcherrima* cannot be described owing to the state of the pressed material.

Transverse sections of parts of the leaves were made by means of a freezing microtome, and the sections were stained with cotton blue. The internal hyphae were observed in the substomatal chambers and occasionally in intercellular spaces of the mesophyll (Fig. 1). No haustoria were seen. Emergence of hyphae through the stomata has been observed in transverse sections, but was seen more clearly on epidermal strips of the host leaves. The strips were placed in lactic acid with cotton blue and examined under a low-power objective without a cover-slip. Up to three hyphae were seen emerging from a single stoma (Fig. 2*e*).

This species is found to resemble *L. taurica* in certain characteristics.

(1) In *L. taurica*, at this late stage of development, the external mycelium is relatively abundant, and so it is in the type material of this specimen. The hyphae branch on the leaf surface with the conidiophores bearing

conidia singly on their tips. Like *L. taurica* at this stage, the hyphae emerging through the stomata only occasionally grow directly into conidiophores (Fig. 2).

(2) The internal hyphae are more or less confined to the subepidermal layer of the mesophyll, rarely occurring deeper and frequently separating the epidermis from the underlying tissue.

Fig. 1. Transverse section of *Euphorbia pulcherrima* leaves infected with *L. clavata* sp.nov. Note concentration of internal hyphae in substomatal chamber. (Camera lucida.)

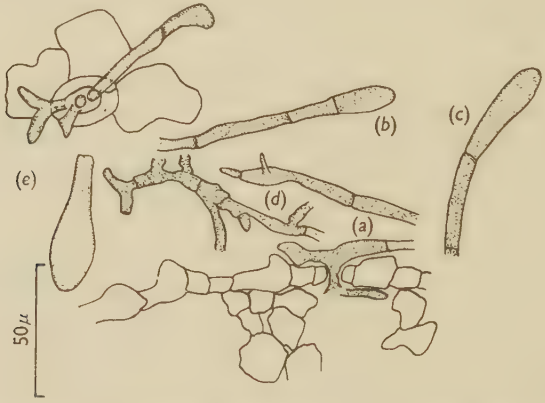

Fig. 2. Transverse section of *Euphorbia pulcherrima*. (*a*) Hypha emerging through stoma; (*b*) immature conidium; (*c*) young conidium; (*d*) superficial hyphae; (*e*) epidermis of *E. pulcherrima*, surface view. Hyphae emerging through a stoma. (Camera lucida.)

(3) The internal hyphae are most abundant within and around the substomatal chamber.

(4) The cleistothecia are borne superficially on the lower surface. They are light brown, becoming dark brown to black at maturity. When young, they are more or less spherical. The mature cleistothecia, however, are hemispherical, lenticular or pezizoid but not spherical as in the other members of the Erysiphaceae. This is also one of the main features of the monotypic genus *Leveillula* (Arnaud, 1919), the type species (*L. taurica*) being described earlier by Léveillé (1851) as *Erysiphe taurica* Lév. (Saccardo, 1882). The cleistothecium is irregularly covered with numerous

Fig. 3. *Leveillula clavata* sp.nov. A, longitudinal section of cleistothecia; B, free ascus and ascospore. (Camera lucida.)

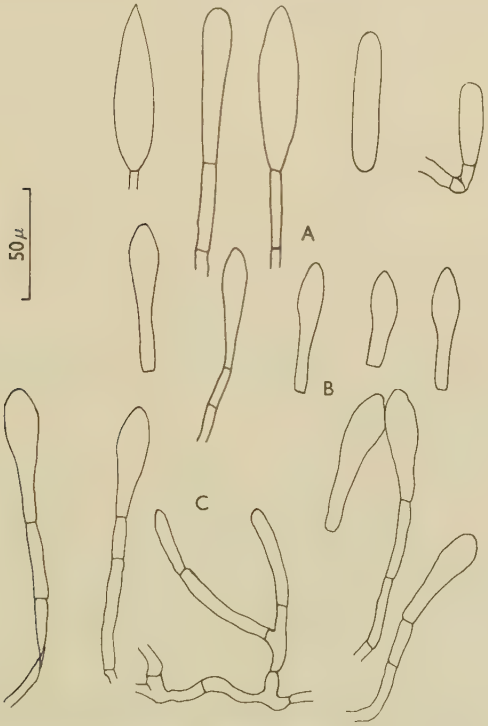

Fig. 4. A, conidia and conidiophores of *Leveillula taurica* (Lév.) Arn.; B, C, *L. clavata* sp.nov. conidia, conidiophores and superficial hyphae. (Camera lucida.)

simple, lax, hyaline appendages without swollen bases (thus differing from *Phyllactinia* spp.), sometimes septate and occasionally branched. The number of asci is numerous (10–30 or more), and each ascus contains two ascospores (Fig. 3).

This species differs from *L. taurica* in certain characters.

(1) The conidium has a distinct club-like shape not unlike that of *Phyllactinia corylea* (Pers.) Karst. It is slender towards the proximal end, becoming abruptly wider one-third to half the way along its length, and broadening distally before finally narrowing into a blunt tip. The shape of the conidia tends to be much more constant in this species than in *L. taurica* where there is usually considerable variation (Salmon, 1906; Arnaud, 1919) even in samples from the same leaf. The conidia of *L. taurica* never assume the form found in this new species (Fig. 4).

(2) The conidia are narrower and, on the average, shorter than those of *L. taurica*.

(3) The cleistothecia, the asci and the ascospores are generally smaller (Table 1).

Table 1. *Measurements* (μ) *of different parts of* L. clavata *sp.nov. and* L. taurica (*Lév.*) *Arn.*

	L. clavata		*L. taurica*	
	Length	Width	Length	Width
Conidia	38–76 (55)	10–16 (*a*) (12) 6–8 (*b*) (6)	40–83 (63)	12–16 (*c*) (14)
Conidiophores	38–40 (38)	6–9 (7)	38–41 (38)	6–9 (7)
Cleistothecia	120–200 (*d*) (180)	80–120 (*e*) (100)	160–240 (*d*) (200)	80–120 (*e*) (100)
Asci	58–65 (60)	20–24 (*c*) (20)	65–100 (80)	18–24 (*c*) (20)
Ascospores	18–26 (20)	12–14 (*c*) (12)	25–40 (30)	12–14 (*c*) (14)

a=at broadest distal region; *b*=at narrowest proximal region; *c*=at broadest region; *d*=diameter; *e*=depth.

The figures in parentheses refer to average dimensions.

From the differences cited above, and especially because of the shape of the conidia, this species is considered to be sufficiently distinct from *L. taurica* to merit specific rank.

I am much indebted to Dr M. B. Ellis for the Latin diagnosis.

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SOME NEW AND INTERESTING SPECIES OF MICRO-FUNGI

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(With Plates 21–23)

Mucor vesiculosus G. Smith, from garden soil, Harrow; *M. petrinsularis* Naumov var. *ovalisporus* G. Smith, from infected human ear; *Penicillium cylindrosporum* G. Smith, aerial contaminant; *P. albo-aurantium* G. Smith, from garden soil, Harrow; *P. radulatum* G. Smith, from heathland soil, many localities; *P. griseolum* G. Smith, from heathland soil, Studland, Dorset; *P. raperi* G. Smith, from cultivated soil, Bedford, are described as new. The zygosporic state of *Mucor pusillus* Lindt is described. A Latin diagnosis is given to validate the name *Pleurage unicaudata* Moreau et Moreau (1955).

Mucor vesiculosus sp.nov. (Pl. 21, figs. 1–3)

Coloniae in agaro 'malt' dicto vel aliis substratis celeriter crescentes, floccosae ad 5 mm. crassae, albae, reverso non-colorato; hyphae ad 7·5 μ diam.; sporangiophori brevi, 2·2–3·5 μ diam., plerumque non ramosi sed aliquando ramosi sympodiales, plerumque cum septo 10–15 μ sub sporangio, ad apicibus vesiculas subsporangias, 7·5–14 μ diam., formantes; sporangia globosa, 15–45 μ diam.; columellae 5–10 μ altae et 8–16 μ crassae; sporae inequaliter ovatae, 2–2·8 × 1·5–1·8 μ.

Habitat in solo hortense, Harrow, Anglia.

The fungus grows well on all the media commonly used in the laboratory, forming floccose colonies about 5 mm. deep, persistently white and with reverse uncoloured; vegetative aerial hyphae long and stout, mostly 6–7·5 μ diam.; sporangiophores arising mostly as short branches from aerial hyphae, 2·2–3·5 μ diam., usually with a septum 10–15 μ below the sporangium, mostly unbranched but occasionally forming sympodial cymes; sporangiophores terminating in a sub-sporangial vesicle, 7·5–14 μ diam.; sporangia more or less globose (exclusive of the vesicle), 16–45 μ diam., columellae hyperhemispherical, 5–10 μ high and 8–16 μ broad, colourless; spores colourless, irregularly ovate, 2–2·8 × 1·5–1·8 μ.

Type culture isolated from garden soil, Harrow, England.

This species is distinguished from all others of the genus by the vesicle-like swelling below the sporangium. It is also peculiar that the colony colour is persistently white, even when abundant sporangia are produced.

The formation of the sporangium begins by the dilation of the tip of the short sporangiophore to form an oval or pear-shaped structure. Soon this shows a slight constriction, usually somewhat below the middle, and a septum appears at this point. Pl. 1, fig. 1, shows a young sporangium just before the septum is formed. Pl. 1, fig. 2, shows a later stage, with the septum complete. The upper cell enlarges to form the sporangium proper, whilst the septum pushes up into the sporangium to become the

columella. The subsporangial swelling and the columella are thus continuous, although they are deliminated by a constriction from which arises the sporangial wall (Pl. 1, fig. 3).

Cultures are maintained in the L.S.H.T.M. collection as BB 321.

Mucor petrinsularis Naumov var. **ovalisporus** var.nov. (Pl. 21, fig. 4)

A *M. petrinsularis* differt propter sporas ovatus, 8–11·5 × 5–8 μ, nunquam globosas. In aure humana inventus.

The fungus was isolated by Dr W. G. Henderson, of the London Hospital, from the ear of a patient complaining of external otitis. A culture was sent to Dr J. Walker, of the Mycological Reference Laboratory, L.S.H.T.M., and was passed on to me for identification. It could not be placed with the help of Zycha's key, which is based primarily on spore shape. However, the description of *M. petrinsularis* Naumov, both as given by Naumov (1939) and by Zycha (1935), fits this species, except for the shape of the spores, which are regularly ovate or ellipsoidal, 8–11·5 × 5–8 μ, i.e. with a length/breadth ratio of approximately 1·5:1, whereas the spores of *M. petrinsularis* are consistently round.

The most characteristic feature of both *M. petrinsularis* and the new variety is the persistence of the sporangial wall. The sporangial membranes of most species of the genus disintegrate completely when mounted in fluid, apparently dissolving or breaking into minute fragments, whereas in this case the wall splits with pressure on the cover-glass, but remains in large pieces, often with one of the pieces remaining attached to the sporangiophore (Pl. 1, fig. 4), according to Zycha 'like dishes containing spores'. The branching of the sporangiophore is also highly characteristic. Irregular sympodia are produced, with each successive branch arising immediately below a septum, which latter is only a very short distance below the previously formed sporangium (Pl. 1, fig. 4).

Cultures are maintained in the L.S.H.T.M. collection as BB 331.

Mucor pusillus Lindt, zygosporic state. (Pl. 21, fig. 5)

In October 1955 I received from Mr P. K. C. Austwick, of the Veterinary Laboratory of the Ministry of Agriculture, Weybridge, a culture labelled '*Mucor pusillus*? with zygospores'. This is one of the two isolates referred to by Ainsworth & Austwick (1955, p. 378).

The culture as received contained very numerous zygospores. Subcultures on a number of different media were very similar, forming a dark turf only about 2 mm. high, with reverse dirty brown. The details of branching and of the sporangial structures and spores agreed well with the diagnosis of *M. pusillus* given by Zycha (1935, p. 51). In all the cultures abundant zygotes were found, always growing very close to the substrate. In order to decide whether the fungus is homo- or heterothallic, a series of dilution cultures was made. All isolates from well-separated colonies, presumably originating from single spores, were alike and produced zygospores freely. In addition, a number of single sporangiospores were isolated. Not all of these grew, but eighteen cultures were obtained, all zygosporic. It may, therefore, be taken that the fungus is homothallic.

The zygotes do not arise from bifurcated hyphae, as is the case with many of the homothallic Mucoraceae, but are formed between hyphae of widely separated origin. One of the hyphae ends in a sterile tip, just beyond the point at which the suspensor arises; the other grows on and may produce one or more further gametangia. Pl. 21, fig. 5, shows a typical zygospore *in situ* in a Petri dish culture. The zygospores are globose or slightly flattened at the sides, very dark almost black, covered with prominent conical warts, 55–75 μ diam. The suspensors are approximately equal, elongated conical.

Hesseltine (1954) erects a section of *Mucor*, Sect. *Genevensis*, to include the homothallic species of the genus. According to the definition of the section it ought to include the fungus described above. However, Hesseltine's key separates first the Sect. *Ramannianus*, then Sect. *Sphaerosporus*, which includes *M. pusillus*, and gives as a character of the Sect. *Genevensis* 'sporangiospores not spherical, or at least only a few spherical'. If the fungus under discussion is to be included in Sect. *Genevensis*, then, not only would it have to be regarded as a new species, but the definition of the section would have to be revised. In my opinion it is preferable to extend the diagnosis of *M. pusillus* to include the zygosporic phase, a preference supported by the common origin of both zygosporic and non-zygosporic strains from similar cases of animal mycosis.

Cultures are maintained in the L.S.H.T.M. collection as BB 325.

Penicillium cylindrosporum sp. nov. (Pl. 22, figs. 1, 2)

Coloniae in agaro Czapekii tenuae, velutinae, sordide avellaneae, reverso incolorato; in agaro 'malt' dicto celeriter crescentes, concoloratae sed margine subflavo, velutinae, reverso atrovirido-brunneo; penicilli asymmetrici, divaricati; conidophora longa, ex substrato, sinuosa, asperula, 2·5–4 μ diam.; rami asperuli, 16–25 × 2·5–4 μ; metulae plerumque 12–20 μ longae, sed 9–10 μ in penicillis complicatis, 2–4 μ diam.; phialides 11 × 2·5–3 μ; conidia plerumque cylindrica, 4·2–5·5 × 1·8–2·2 μ sed aliquando ovata, 3·2–3·5 × 2·2–2·5 μ in catenis implicatis aut in pluribus columnis laxis.

Fungus in culto laboratorio inventus, Harwell, Anglia; leg. Mrs J. Robson.

Colonies on Czapek agar thin velvety, inclined to be patchy, dull avellaneous, with reverse uncoloured; on malt agar growing fairly well, velvety, dull avellaneous with sordid yellow margin and with reverse dark dirty greenish brown; on potato agar similar except that the reverse is somewhat more greenish; stated by Mrs J. Robson (from whom the original culture was received) that green respiration drops are produced in old cultures, but these have not been observed in subcultures; penicillus sometimes with a more or less divergent cluster of metulae (the *P. citrinum* type), but more often irregular, with a branch or branches from lower nodes, bearing phialides alone or metulae and phialides mixed in the same verticil, sometimes showing the same mixed verticils in the terminal part of the structure; conidiophores long and sinuous, mostly arising from submerged mycelium, rough, 2·5–4 μ diam.; rami, when present, rough, 16–25 × 2·5–4 μ; metulae usually 12–20 μ long, but shorter, 9–10 μ, in the more complicated heads, and 2–4 μ diam.; phialides almost cylindrical, tapering abruptly at the tip, very regular in length, 11 × 2·5–3 μ; conidia usually cylindrical (hence the specific epithet), 4·2–5·5 × 1·8–2·2 μ, mostly 5 × 2 μ, but occasionally ovate to ellipsoidal, 3·2–3·5 × 2·2–2·5 μ; conidial

chains divergent then tangled, or sometimes in several loose, twisted columnar masses.

Description based on a culture sent for identification by Mrs J. Robson, of the Atomic Energy Establishment, Harwell, who found it as a Petri dish contaminant.

This species is to be classified in the Asymmetrica-Divaricata (Raper & Thom, 1949), and is best placed, at least for the time being, in the *P. lilacinum* series, which includes the species which entirely lack green colour in the conidia.

Cultures are maintained in the L.S.H.T.M. collection as BB 326.

Penicillium albo-aurantium sp.nov. (Pl. 23, fig. 1)

Coloniae in agaro Czapekii vel agaro 'malt' dicto, vel agaro cum decocto tuberorum, similes, sublente crescentes, floccoso-funiculosae, tenuiores ad marginem, constanter albae, reverso flavo dein aurantio; penicilli divaricati compacti vel laxi, plerumque ter verticillati; conidiophora glabra, 30–400 μ longa et 2–2·5 μ diam.; metulae plerumque 4–6 × 2–2·2 μ sed aliquando ad 10 μ longae; phialides vulgo 5 × 2 μ sed interdum ad 7–8 μ longae; conidia ovata vel citriformia, glabra, 2·3–2·5(3) × 1·5–1·8 μ, in catenis implicatis.

Habitat in solo hortense, Harrow, Anglia.

On Czapek agar, malt agar, or potato agar grows somewhat slowly, about 20 mm. diam. in 7 days at 24° C., but eventually spreads broadly, floccose funiculose, fairly thick in the centre and thinning towards the margin, persistently white but sometimes appearing yellowish from the bright reverse colour showing through; reverse deep yellow to bright orange; penicillus typically divaricate, sometimes consisting of a fairly compact, thrice verticillate, terminal structure, with numerous metulae and phialides, but often very irregular and loose in structure; conidiophores smooth, very variable in length, 30–400 μ, and 2–2·5 μ diam.; metulae mostly 4–6 × 2–2·2 μ, but occasionally up to 10 μ long; phialides in most penicilli very regular in size, 5 × 2 μ, but sometimes 7–8 μ long, tapering fairly abruptly at the tip; conidia ovate to lemon-shaped, smooth, 2·3–2·5, rarely 3, × 1·5–1·8 μ, in chains which soon become much tangled.

This species, on account of the structure of the penicilli and the texture of the colonies, is to be classed in the Asymmetrica–Divaricata, and, like *P. cylindrosporum*, it will have to be placed, for the time being, in the *P. lilacinum* series, simply on account of its total lack of green colour. However, it shows little similarity to *P. lilacinum Thom*, or *P. humuli* van Beyma, and still less to *Spicaria violacea* Abbott (= *Paecilomyces marquandii* (Massee) Hughes).

The specific epithet refers to the colours of surface and reverse, white and orange.

The fungus was isolated from garden soil near Harrow, Middlesex. Cultures are maintained in the L.S.H.T.M. collection as BB 327.

Penicillium radulatum sp.nov. (Pl. 22, figs. 3, 4)

Coloniae in agaro Czapekii late crescentes, cum margine aequaliter rotunda, venetae dein fumoso-venetae, velutinae, guttulis minutis flavis, reverso virido-flavo, pigmento in agaro differente; in agaro 'malt' dicto aut agaro cum decocto tuberorum late

crescentes, fumoso-venetae, velutinae, guttulis minutis flavis, reverso flavo dein aurantio pigmento aliquatenus differente; penicilli inaequaliter divaricati, laxi, omnino asperi; conidiophora asperissima, 2·2–3·5 μ diam; rami et metulae saepe similes, inconstantissimi in longitudine, asperi; phialides asperae, 8–9 × 2–2·4 μ; conidia globosa, spinulosa, plerumque 2·8–3·2 μ interdum ad 4·5 μ diam; catenae conidiorum plerumque implicatae sed aliquando in columnis laxis.

Habitat in solo, socia *Calluna*, Surrey, Dorset et Norfolk, Anglia; Glamorganshire et Anglesey, Cambria; leg. G. W. F. Sewell.

Colonies on Czapek agar spreading broadly, circular with regular margins, dark bluish green with narrow paler edge during the rapidly growing period, becoming very dark smoky blue-green, smooth velvety; respiration drops minute, yellow; reverse somewhat greenish yellow, with the colour diffusing throughout the agar; on malt agar or potato agar spreading fairly rapidly, 23–30 mm. in 9 days at 24° C., dark greyish blue-green with paler bluish margin, velvety; drops minute, yellow; reverse yellow to orange or reddish orange, with the colour diffusing slightly; penicilli markedly divaricate with the branches loosely divergent, usually with a terminal cluster of metulae and phialides mixed, and with a series of branches from lower nodes, bearing metulae and phialides or phialides alone; conidiophores arising from the substrate, very rough, fairly long and 2·2–2·5 μ diam.; rami and metulae often indistinguishable and very variable in length, rough; phialides rough, fairly abruptly pointed, 8–9 × 2–2·4 μ; conidia globose, spinulose, mostly 2·8–3·2 μ diam. but occasionally up to 4·5 μ, in chains which are usually divergent at first, becoming tangled, but are sometimes more or less parallel or even in loosely columnar masses.

The isolate on which the description is based was sent for identification, in March 1954, by Mr G. W. F. Sewell, of the Royal Holloway College, as 'E62, from *Calluna* heathland soil'. Mr Sewell stated, in a more recent letter, that he has no record of the actual locality from which this isolate was collected but that the same species was found in *Calluna* heathland soils from Puttenham Common and Chobham Common, Surrey; Newborough, Anglesey; Godlingston Heath and Plateau Heath, Dorset; Kelling Heath, Norfolk; and Rhossilly Downs, Glamorganshire.

Species of *Penicillium* in which both conidiophores and conidia are definitely rough are few in number, and I know of no other species which has all parts of the penicillus, including even the phialides, as well as the conidia, markedly asperulate. The fungus is to be classed in the Asymmetrica–Divaricata, and is closest to *P. melinii* Thom, which also has rough conidiophores and echinulate conidia. It differs from the latter in colony colour and in the more constantly divaricate character of the penicillus. The epithet is from Latin *radula* = a scraper.

Cultures are maintained in the L.S.H.T.M. collection as BB 333.

Penicillium griseolum sp.nov. (Pl. 23, fig. 2)

Coloniae in agaro Czapekii tenuissimae, translucidae, vix tandem coloratae; in agaro 'malt' dicto sublente crescentes, primum subgriseae deinde atrogriseae, funiculosae, densae, circa 1 mm. crassae, reverso virido-brunneo; penicilli monoverticillati; conidiophora brevissima, ex hyphae aetheriis, glabra, 12–35 × 1·2–2 μ; phialides paucae in verticillo, 6–7 × 1·6–2 μ; conidia matura globosa vel subglobosa, subverrucosa, 3·1–4 μ

diam., plerumque 3·2–3·5 μ, conidia juvenica parva, cylindrica, oidiis similia, tarde decidua; catenae conidiorum parallelae vel in columnis digestae.

Habitat in solo litorale, Studland, Dorset, Anglia; leg. Juliet C. Brown.

Colonies on Czapek agar spreading moderately quickly but extremely thin, transparent, showing no visible mycelium but only scattered penicilli, giving a very faint greenish hue to the colony; on malt agar or potato-dextrose agar growing moderately well, at first pale grey with broad white edge, turning darker grey, matted funiculose about 1 mm. thick; reverse brown with a greenish tone (somewhat like mustard but rather darker and duller); penicilli strictly monoverticillate; conidiophores arising from aerial hyphae, very short, smooth, 12–35 × 1·2–2 μ; phialides few in the verticil, abruptly pointed, 6–7 × 1·6–2 μ; ripe conidia globose or nearly so, faintly verrucose, 3·1–4·0 μ diam. but mostly in the range 3·2–3·5 μ, in parallel chains or forming somewhat loose columns; young conidia oidium-like, small, cylindrical, swelling only slowly and remaining in chains attached to the phialides, even on mounting in fluid.

The isolate on which the description is based was received in July 1955 from Miss Juliet C. Brown, of the Royal Holloway College, as 'D476, from the acid dunes of Studland, Dorset'.

This species belongs to the Funiculosa section of the Monoverticillata, in the *P. adametzi* series, and closest to *P. terlikowskii* Zaleski, from which it differs in its very poor growth on Czapek agar, in its lack of green colour on malt agar, in its different reverse colour, and in the curious tendency, conspicuous otherwise only in *P. italicum*, to form oidium-like chains of very slowly ripening conidia.

Cultures are maintained in the L.S.H.T.M. collection as BB 323.

Penicillium raperi sp.nov. (Pl. 23, figs. 3, 4)

Coloniae in agaro Czapekii restrictae, floccosae, compactae, primum subluteae dein inaequaliter subluteae submalvaceaeque, demum subglaucae, guttulis parvis incoloratis, reverso cinnamomeo cum margine lutea; coloniae in agaro 'malt' dicto aliquatenus celeriter crescentes, subluteae, conidia lentissime gerentes, reverso cinnamomeo; coloniae in decocto tuberorum cum agaro subceleriter crescentes, primum subluteae, dein zonatae, submalvaceae, subglaucae, subluteaeque, reverso primum cinnamomeo cum margine lutea demum medio purpureo; penicilli monoverticillati vel inaequaliter ramosi; conidiophora brevissima, ex hyphis aetheriis, 15–25 × 1·3–1·5 μ; phialides 7–9 × 1·8–2·2 μ, paucae in verticillo; conidia inaequaliter ovata, subaspera, 3–3·5 × 2·4–2·8 μ; catenae conidiorum breves, diversae vel implicatae; hyphae steriles, conidiophora abortiva similes, copiosae.

On Czapek agar grows restrictedly, 22–25 mm. in 9–10 days, and thereafter spreading only slightly, matted floccose about 1 mm. thick, at first pale yellow becoming mauve in patches, tardily turning pale bluish green all over or almost all over, with drops small, colourless, and reverse dull brown with intense yellow edge, but with no diffusion of the pigment into the agar, sporing tardily first in the mauve areas, more freely when the greenish colour appears; on malt agar grows rather more quickly, 30 mm. in 9 days and 42–45 mm. in 15 days, pale yellow and whitish with only traces of pink or mauve and turning greenish only very slowly and incompletely, with reverse deep dull yellowish brown; on potato-dextrose agar grows rather more quickly than on Czapek, much as on malt agar,

yellow at first then with mauve centre but with a middle zone of pale bluish green, with reverse at first brown with yellow margin, then with centre purple and margin zoned with brown and deep greenish yellow; penicilli monoverticillate or, more often, irregular, with two to three unequal metulae, or with various superimposed verticils consisting of both metulae and phialides, but with the number of elements at each stage very small; conidiophores arising from aerial hyphae, very short, mostly less than 25 μ long and 1·3–1·5 μ diam.; phialides few in the verticil, usually divergent, 7–9 × 1·8–2·2 μ, narrowed at the base and with fairly long tip; conidia irregularly ovate, slightly rough, 3–3·5 × 2·4–2·8 μ, many appearing to be globose or subglobose, but these probably ovate conidia seen end-on; conidial chains short, divergent or tangled; numerous sterile branches produced from aerial hyphae, sometimes short and spur-like, sometimes longer, more or less club-shaped and frequently nodulose at the tip, resembling abortive conidiophores.

Isolated from the soil of a cabbage field near Bedford, September 1952. Cultures are maintained in the L.S.H.T.M. collection as BB 338.

A culture of this species was sent, in October 1953, to Prof. K. B. Raper. He reported that he did not believe it to correspond to any species described in the *Manual* (Raper & Thom, 1949), and that it is best placed in the *P. lividum* series, near to *P. aurantio-violaceum* Biourge. With this placing I cannot agree. The key to the Monoverticillata (*Manual*, pp. 127–132), after separating the perithecial and sclerotial species, classifies the rest according to colony texture. There are three main groups, respectively velvety, appearing velvety but with conidiophores as branches from aerial hyphae, and definitely floccose or funiculose. The velvety section includes the *P. lividum* series, and yet this series is further subdivided according as colonies are 'deeply lanose' or not. Apparently Raper & Thom use the term 'lanose' here to describe colonies in which very long conidiophores arise from the submerged mycelium or from a compact basal felt. However, later in the *Manual* (p. 419) the Asymmetrica–Lanata group is described as having 'mycelium consisting of a cottony, lanose, or floccose mass', the term 'lanose' being used here in its usual sense. Obviously, the *P. lividum* series should be described by some other term, since 'lanose' is so misleading. *P. raperi* differs sharply from the species of the *P. lividum* series in having very short conidiophores arising from aerial hyphae, and is more nearly related to the *P. restrictum* series, which is characterized by floccose, not appreciably funiculose, colonies, with conidiophores 'arising primarily from aerial hyphae', and also by the production of spur-like sterile branches. However, the fact that a large proportion of the penicilli are not strictly monoverticillate, but consist of irregular verticils of phialides and metulae, suggests affinity with the *P. janthinellum* series.

Named for Prof. K. B. Raper. Cultures are maintained in the L.S.H.T.M. collection as BB 338.

Pleurage unicaudata Moreau et Moreau in *Bull. Soc. bot. Fr.* **102**, p. 124, 1955

Amongst a batch of unidentified cultures received in 1948 from Mr A. L. James of the Timber Research Laboratory, Transvaal Chamber of Mines,

Johannesburg, was a species believed at the time to be a *Sordaria*. It had been isolated from the interior of an ammunition box in a gold mine. On acquiring Moreau's monograph on *Sordaria* and *Pleurage* (1953) it was apparent that the fungus was to be classed in *Pleurage sensu* Moreau, but equally apparent that it did not fit with any of the described species. In March 1955 I sent a culture to Dr C. Moreau, stating that it appeared to be unidentifiable from his keys. A description of the fungus, as *Pleurage unicaudata* n.sp., was subsequently published by Dr and Mme Moreau, but without a Latin diagnosis. In order to validate the name I append a Latin diagnosis, substantially in the terms of the original French diagnosis.

Pleurage unicaudata Moreau et Moreau

Perithecia superficialia, pyriformia, flavo-brunnea, collis magnis, 250–500 × 150–300 μ; asci cylindrici vel claviformes, pedicellati, 140–160 × 20–26 μ; sporidia biseriata, limoniformia, brunnea, 17–29 × 13–15 μ, cum una appendice primaria in basi et appendicibus secundariis absentibus.

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EXPLANATION OF PLATES 21–23

Plate 21

Fig. 1. *Mucor vesiculosus*. Commencement of delimitation of sporangium. × 1000.
Fig. 2. *M. vesiculosus*. Young sporangium with subsporangial vesicle. × 1000.
Fig. 3. *M. vesiculosus*. Columella and remains of sporangial wall. × 1000.
Fig. 4. *M. petrinsularis* var. *ovalisporus*. Typical branching and fragmentation of sporangial wall. × 200.
Fig. 5. *M. pusillus*. Zygospore. × 250.

Plate 22

Fig. 1. *Penicillium cylindrosporum*. Typical penicilli. × 500.
Fig. 2. *P. cylindrosporum*. Large penicillus. × 500.
Fig. 3. *P. radulatum*. Typical branching. × 500.
Fig. 4. *P. radulatum*. Simple penicilli. × 1000.

Plate 23

Fig. 1. *P. albo-aurantium*. Typical penicillus. × 1000.
Fig. 2. *P. griseolum*. Penicilli. × 1000.
Fig. 3. *P. raperi*. Penicilli. × 1000.
Fig. 4. *P. raperi*. Abortive conidiophore. × 1000.

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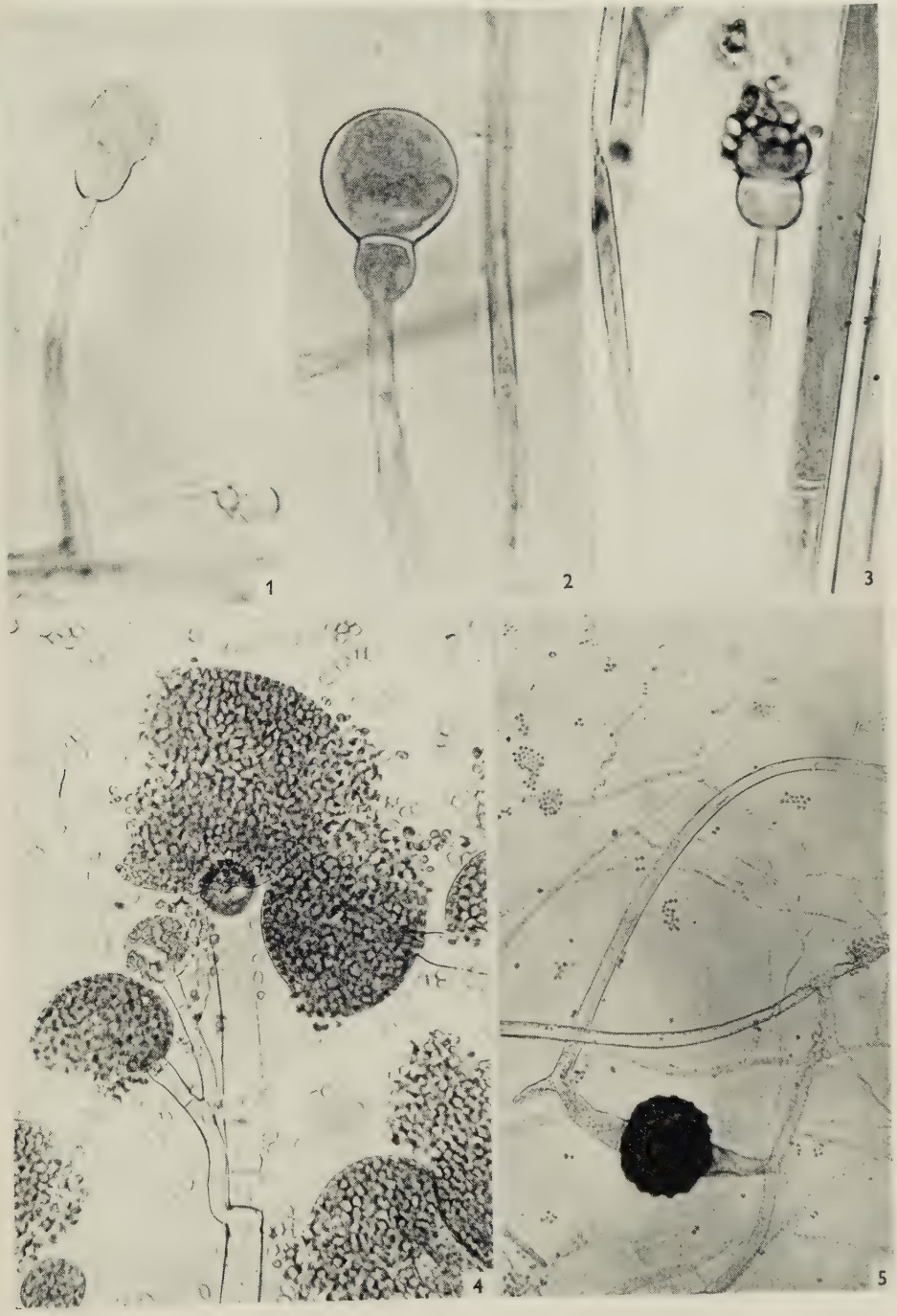

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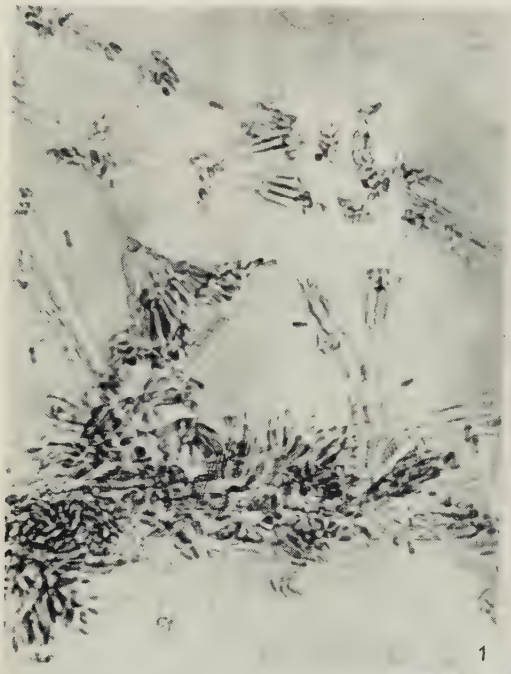
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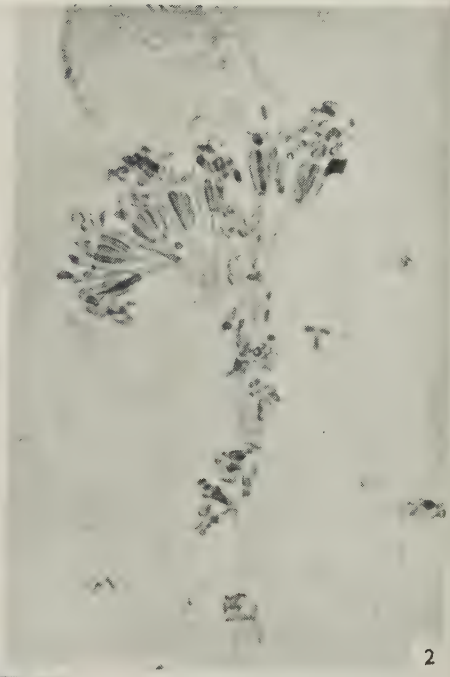
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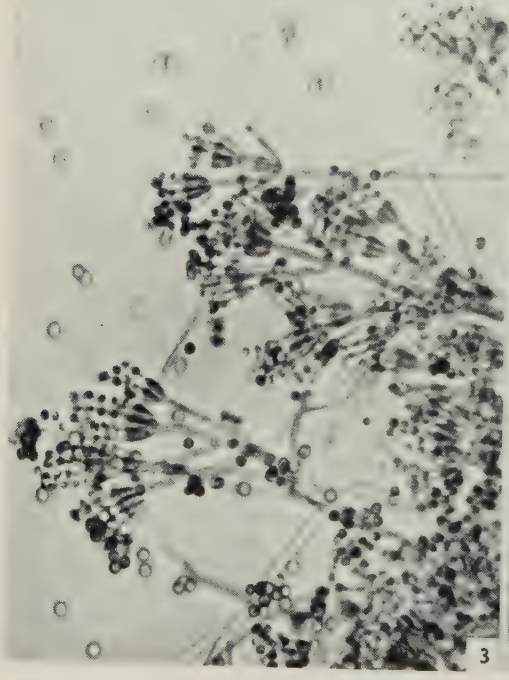
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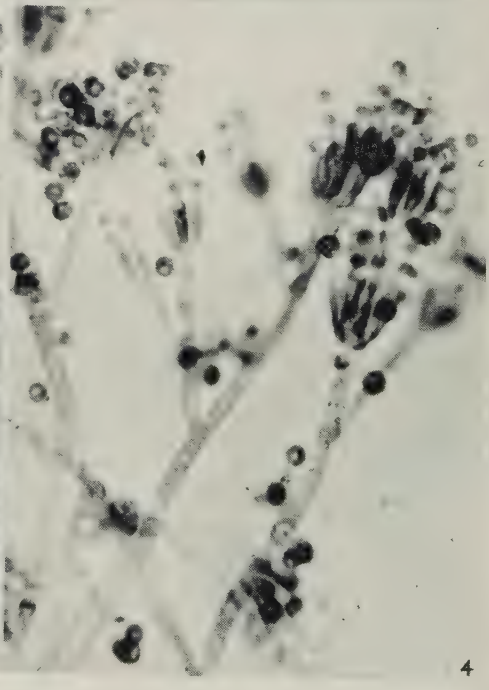
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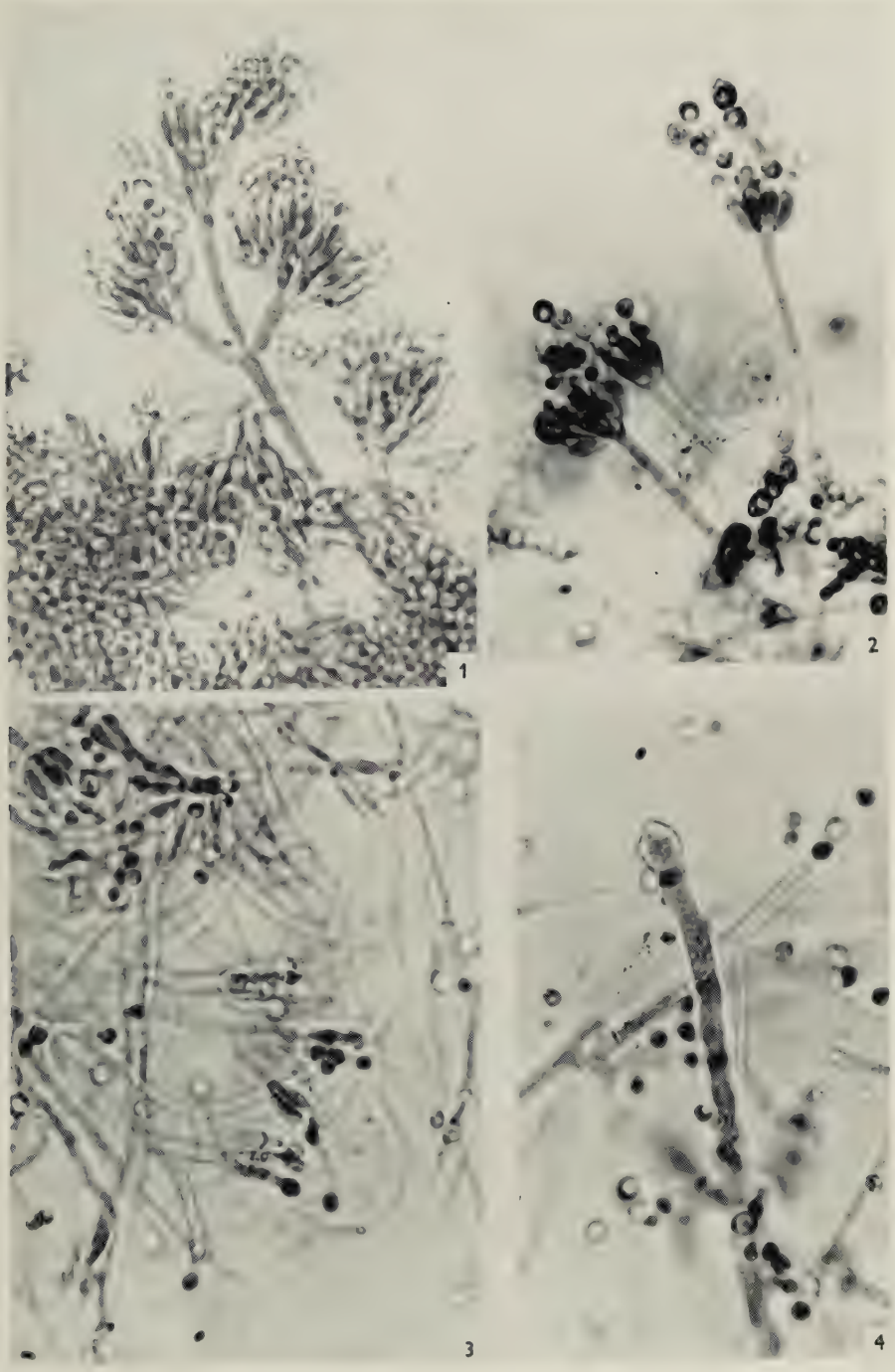
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ON THE PARASITISM OF *SCLEROTINIA TRIFOLIORUM* BY *CONIOTHYRIUM MINITANS*

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Coniothyrium minitans Campb. is pathogenic to *Sclerotinia trifoliorum* Erikss., the causal agent of clover rot. It is recorded for the first time in England and its natural distribution on sclerotia of *S. trifoliorum* is studied. Experiments are described on artificial infection of buried sclerotia by *C. minitans* applied to soils as spore suspensions or mixed with them as cornmeal-sand preparations. A cornmeal-sand culture, mixed intimately with either a loamy sand or a heavy clay, killed 85–99 % sclerotia within 11 weeks. The soils remained highly infective for at least 14 months. The possibilities of *C. minitans* for control of clover rot are discussed.

Introduction

This investigation developed out of an inquiry into the fate of sclerotia of *Sclerotinia trifoliorum* in soil. *S. trifoliorum* Erikss. is responsible for the disease of herbage legumes known as clover rot, a general account of which has been given by Dillon Weston, Loveless & Taylor (1946). The fungus persists from year to year as sclerotia formed on the root system and lower parts of infected plants, which may be killed, and also in the soil nearby. When a field containing these plants is ploughed-up, the sclerotia are dispersed throughout the soil; their future behaviour depends upon depth of burial. They will germinate only if buried above a critical depth of about $2\frac{1}{2}$ in. Germination is seasonal, and results in the production of apothecia, which appear from September to December, but in mild weather this period may extend to about March. Above the critical depth, some of the sclerotia germinate in the autumn of the year in which they formed, others remain dormant and probably germinate in a succeeding year. Sclerotia buried below the critical depth persist for a period of the order of 8 years, during which they will be capable of germination should cultivations bring them to the surface. Farming experience has shown that it is generally safe to sow a crop of clover on land which bore a diseased crop about 8 years previously, and this period may coincide with that required for buried sclerotia to die out. This has never been directly proved. Pape (1937) showed that sclerotia planted below the critical depth were mostly viable after $7\frac{1}{2}$ years.

If the deeper buried sclerotia do not germinate, they must eventually decay. No evidence is available as to the cause of their death. Under certain waterlogged conditions they may be asphyxiated; this occurs in wet heaps of manure (Dillon Weston *et al.* 1946) and in flooded soil (Moore, 1949). Pathogenic micro-organisms may be responsible for the death of some sclerotia and larger organisms, such as slugs, may reduce the numbers. Attempts to destroy sclerotia by biological means have been

reported from Scandinavia. Pohjakallio, Salonen & Relander (1953), in Finland, stated that certain isolates of *Mucor* and *Fusarium* would kill sclerotia on the surface of sterile sand. From Norway, Röed (1954) reported *Mitrula sclerotiorum* Rostr. to be associated with sclerotia and probably parasitic. In 1947, Campbell isolated a new species of *Coniothyrium* from sclerotia of *S. sclerotiorum*, which he named *C. minitans*. The sclerotia, collected from diseased guayule plants, had been plated, and the *S. sclerotiorum* isolates had grown out and formed sclerotia in culture. Some of these later became covered with pycnidia of the *Coniothyrium*. Campbell established that the sclerotia could be attacked by *C. minitans* when buried in sterile sand, either if *C. minitans* were added to the sand as a spore suspension or if the sclerotia were first dipped into a spore suspension and then buried.

A fungus identified as *C. minitans* Campbell was isolated from a culture of *S. trifoliorum*, obtained from sclerotia collected from a clover field near Tillingham in Essex, in the same way as Campbell made his isolation. Since this appeared to be a promising parasite, a study of its relationship with *S. trifoliorum* was undertaken.

Host-parasite relations

In addition to being pathogenic to cultures of *S. trifoliorum*, the Tillingham isolate attacked cultures of *S. sclerotiorum* Lib. de Bary and *S. minor* Jagger, but not those of *S. gladioli* Drayton and *Botrytis fabae* Sard. If *S. trifoliorum* and *C. minitans* be inoculated at opposite sides of a plate of potato-dextrose or potato-sucrose agar, the colonies grow straight towards each other without any signs of inhibition; on meeting, the *Coniothyrium* grows slowly through the *Sclerotinia*, forming pycnidia almost exclusively on the sclerotia of the latter. *S. trifoliorum* has the more rapid growth rate, and, if the two fungi be plated together, will cover the plate and form sclerotia, which are eventually invaded by *C. minitans*. In soil, sclerotia are attacked and the mycelium of *C. minitans* grows through the sclerotial tissue with little apparent disturbance. No defence barrier nor visible line of demarcation can be found between invading hyphae and sclerotium hyphae. Pycnidia are often produced on the exterior of a sclerotium which is only partially infected, and eventually the whole surface may be pimpled with them. Later the invaded sclerotium becomes attacked by secondary parasites and disintegrates. The pycnidia also disintegrate but, even in the last stages of decay, the persistence in numbers of its characteristic oval, brown spores proves *C. minitans* to have been the original pathogen. On apothecia, infection begins as brown spots which enlarge and turn black. The apothecia remain functional for some time before becoming destroyed.

There are several methods of detecting *C. minitans* in sclerotia, the choice of which depends on the extent of invasion. With newly infected sclerotia it is necessary to plate out portions of the interior on to a culture of *S. trifoliorum*. When pycnidia have developed, the sclerotia may simply be placed in water, when spores will exude and form brownish green patches around the sclerotia. On artificially infected sclerotia, after a few weeks

in soil, pycnidia are usually clearly visible to the naked eye, but are less obvious on sclerotia infected naturally in the field. Where a sclerotium is extensively decayed and soft, destruction of the pycnidia prevents exudation of spores, but examination under the $\frac{1}{6}$ objective will reveal numbers of oval, brown spores, $4–6 \times 3–5\,\mu$ in size. For confirmation, the debris may be plated on to a culture of *S. trifoliorum*. It is also possible to determine the presence of *C. minitans* in soil by plating a little of the soil on to a *Sclerotinia* culture.

Distribution of *Coniothyrium minitans*

A survey of the distribution of *C. minitans* in the southern half of England was made during the three years 1953–5, with the kind assistance of the National Agricultural Advisory Service. The data for 1953 are detailed in Table 1 and those for 1954 and 1955 are summarized at the bottom of the table. Both a plating method and a water test for pycnidia were used in 1953 for detection of *C. minitans* in the samples. In the plating method, the sclerotia were surface sterilized in 0·5 % sodium hypochlorite for 20 min., blotted dry and a piece from the interior of each put on to a test-tube culture of *S. trifoliorum*. To record the maximum numbers of infected sclerotia, it was necessary to incubate (at 20° C.) for about a month. For the water test, the sclerotia were placed in Petri dishes of water for an hour and the region around the sclerotia then examined for patches of exuded spores. A direct comparison of the two methods is shown in the middle section of Table 1. The water test was done several days after the plating test. More *C. minitans* was detected by plating out. In the infected samples, 20 % sclerotia (45 out of 233) were assessed as parasitized by plating, whereas 15 % (34 of 233) were recorded as such by the water test. The number of samples which were found to be infected (ten) was the same with either method. Although the water test does underestimate numbers of infected sclerotia, it is much more rapid and convenient and was considered adequate for routine testing of samples in 1954 and 1955.

In 1954, only one sample out of eighteen obtained from widely separated places from the southern half of England was infected; 5 % of the sample was parasitized. Samples were taken from the following counties: four each from Warwicks and W. Suffolk, two each from Staffs, Monmouth, Hants and Beds, and one each from Oxon and Cambs. All the samples were collected in April, except those from Suffolk, which were obtained in July.

In 1955, samples were collected in April and May from the following counties: Suffolk (9), Beds (4), Essex (4), Herts (3), Hunts (2) and one each from Lincoln, Worcs and Cambs. Two of the infected samples came from Suffolk and two from Essex, and *c.* 10 % of these samples were parasitized.

C. minitans was found quite frequently in the eastern counties, Cambs, Norfolk, Suffolk and Essex, where, of 39 samples, 15 were infected, whilst of 31 other samples only 1 (from Beds) was infected. There was no observable relation between soil type and *C. minitans*. Infected sclerotia were found on both light and heavy soils.

Table 1. *Infection of naturally occurring sclerotia of* Sclerotinia trifoliorum *by* Coniothyrium minitans

Dates (all in 1953)	Locations	Sclerotia: No. infected by *C. minitans*: Plating method	Sclerotia: No. infected by *C. minitans*: Water test	Sclerotia: No. in sample
13 April	W. Suffolk			
	Alpheton	7	—	31
	Kersey	2	—	12
	Withersfield	0	—	11
20 April	Norfolk			
	Docking	1	—	16
	Gooderstone	0	—	12
22 April	Bedfordshire			
	Everton	0	—	12
	Luton	0	—	11
	Tempsford	0	—	16
24 April	Cambridgeshire			
	Cambridge	0	—	27
19 May	Norfolk			
	Barford	0	0	19
	Diss	0	0	12
	Wramplingham	0	0	15
28 May	Essex			
	Epping	0	0	10
	E. Suffolk			
	Badingham	0	0	51
	Eye 1	6	5	14
	Eye 2	2	1	3
29 May	Cambridgeshire			
	Trumpington	14	9	34
5 June	Fen Ditton	1	1	52
12 June	W. Suffolk			
	Fornham St Martin	14	13	33
	Ixworth 1	4	4	33
	Ixworth 2	0	0	13
	Stowlangtoft	0	0	59
	Coney Weston	0	0	32
27 June	Bedfordshire			
	Stagsden	4	1	64
	Pavenham	—	0	45
	Pertenhall	—	0	22
	Thurleigh	—	0	73
Totals detected by each method where both used		45	34	444

	Sclerotia: No. infected by *C. minitans*: Plating method	Sclerotia: No. infected by *C. minitans*: Water tests	Sclerotia: No. in samples	Sclerotia: Proportion of samples infected
Totals, 1953	55	34	732	10/27
Totals, 1954	—	3	734	1/18
Totals, 1955	—	16	858	4/25

Experiments on artificial infection of buried sclerotia

With spore suspensions as inoculum

In a preliminary experiment, sclerotia obtained from cultures on sterilized carrots were buried in small glazed pots of loamy sand from the Cambridge University Farm. Twenty sclerotia were buried in each pot, in holes pushed into the soil by a twenty-pronged planting device. Soil was then pushed over to fill the holes. *C. minitans* was applied as a spore suspension. The moisture content of the soil was adjusted once weekly to 60% moisture holding capacity (M.H.C.), by which time it had fallen to 15–30% M.H.C. A fluctuating moisture content was considered to approach more closely to field conditions than a constant one. Experimental treatments were applied to duplicate pots; each infection percentage was thus assessed on forty sclerotia. Two pots were kept at winter temperatures (from January to March 1953). The rest were put into a heated glasshouse, at some 5–10° C. above outside temperatures. The effects of varying depth of burial of the sclerotia and concentration of the spore suspension inoculum are summarized in Table 2.

Table 2. *Effect of environmental conditions on infection of sclerotia by* Coniothyrium minitans *after 7 weeks*

Depth of sclerotia (in.) (at spore concentration 'S')			0·5	1·0	1·5	
Percentage infected sclerotia			81	66	8	
Spore concentration S* (at 1 in. depth)	S	0·1 S	0·01 S	0·001 S	0·0001 S	0
Percentage infected sclerotia	66	68	40	33	13	10
Environment (sclerotia at 1 in. at spore concentration 'S')		Glasshouse			Outside	
Percentage infected sclerotia after 7 weeks		66			0	
Percentage infected sclerotia after 13 weeks		—			80	

* S = Twenty ml. spore suspension containing two million spores per ml.

These results showed that spore suspensions added to soil caused considerable infection. Percentage infection was reduced both with increasing depth of burial of sclerotia and with decreasing concentration of *C. minitans* spores below 0·2 million/ml. Low outside temperatures either prevented infection or so reduced the rate of growth of *C. minitans* inside the sclerotia that no external signs of infection were evident after 7 weeks. After a further 6 weeks, of March and April temperatures, there was high percentage infection.

In the summer, an opportunity arose to test the action of spore suspensions of *C. minitans* on sclerotia occurring naturally, after a heavy attack of clover rot in a field of medium-loam soil at Fen Ditton near Cambridge. Some natural parasitism had been recorded from a very large sample of sclerotia taken from this field in late June before the experiment was begun, eighteen sclerotia being found infected out of a total of 870. On bare patches consisting of rows of dead clover plants, twenty separate

square-yard plots were marked out in July, and spore suspensions equivalent to those used in the pot experiments were applied. The result was entirely negative; of 1694 sclerotia dug from the twenty plots in September 1953, only eight were infected. Separate tests showed these sclerotia to succumb to infection under pot conditions, so that the sclerotia were not resistant. There was no shortage of moisture during the initial period of the trial. It was suspected that the reason for the failure of this inoculation was that the heavier and undisturbed soil had filtered off the spores at the surface.

Another experiment was set up in July 1953 to study the effect of depth of burial of sclerotia on infection by *C. minitans*, with observations on apothecial production and persistence of sclerotia. Sclerotia from carrot cultures were buried at 1, 2, 4 and 8 in. in three soils and in sand, respectively, contained in a number of 4 × 12 in. drain-pipes. During preparation, the pipes were filled to planting depth with soil, the sclerotia were put in position and covered with 1 in. soil. 20 ml. of a suspension of *C. minitans* spores, containing two million spores per ml., were then poured on to each pipe, so that, irrespective of depth, the sclerotia in each soil type would be inoculated with the same number of spores. The pipes were then completely filled with the various soils and buried vertically in a light soil at the University Farm, so that the tops of the pipes were flush with the surface of the field. Three soils from the University Farm were used in the pipes: loamy sand from the Gravel Pit coarsely sieved to remove stones; medium-heavy loam from Coprolite Field, similarly sieved; and Gault clay from Charity Field, taken from between rows of beans and in excellent tilth. This was not sieved.

The pipes were inoculated on 24 July 1953. Observations were made on the number of apothecia produced after 4–6 months, and on the number of sclerotia which remained after 15 months. These are recorded in Table 3. Counts of apothecia were made on five occasions between 6 November 1953 and 14 January 1954, the maximum count and the date thereof being recorded in the table.

Large numbers of apothecia were produced from sclerotia buried at 1 and 2 in. in the three soils and a smaller number in sand. This indicated little parasitism of sclerotia in the soils. Many apothecia became infected in the soils and in sand and were finally destroyed, though they remained in functional condition for some time before destruction. The stipes probably became infected on passing through the soil. The development of apothecia in sand was poorer than in the soils, the disks failing to expand properly.

After 15 months few sclerotia were recovered from the top inch of the soils, many of them having previously germinated. Rather more were found in sand, from which fewer apothecia had emerged. Only in sand was it possible directly to detect *C. minitans* on sclerotia after 15 months. The infected sclerotia were completely disintegrated, leaving a few particles of rind and a blackish green stain of *C. minitans* spores in the sand. It was noted that there was no lateral spread of these spores to healthy sclerotia not more than an inch away during this long period, though there was free access to rain and insects. From 2 in., more sclerotia were

recovered, and at 4 and 8 in. most of them had persisted. Some sclerotia, especially those from sand, were developing apothecial stipes, showing that dormancy was breaking after a year's burial. No germination tests were done on the others; they were clean and obviously alive, or decomposition would have set in. There was no sign of decay through anaerobic conditions, though one would expect little oxygen to be present during wet weather at 8 in. down in the Gault clay.

Table 3. *Persistence and germination of sclerotia of* Sclerotinia trifoliorum *buried at four depths in soils and sand*

Depth of burial (in.)	Soil type	Apothecia*			Sclerotia	
		Produced in same season (1953–4)		Stipes noted in next season (Oct. 1954)	Percentage recovered ungerminated Oct. 1954	Percentage parasitized by Oct. 1954
		No.	Date			
1	Sand	57	12 Dec.	2	26	30
	Loamy sand	209	6 Nov.	0	4	—
	Medium loam	178	12 Dec.	0	7	—
	Clay	172	6 Nov.	0	9	—
2	Sand	6	14 Jan.	28	42	22
	Loamy sand	142	12 Dec.	8	31	—
	Medium loam	164	19 Dec.	0	11	—
	Clay	89	19 Dec.	0	70	—
4	Sand	0	—	61	89	6
	Loamy sand	0	—	6	92	—
	Medium loam	0	—	6	95	—
	Clay	0	—	0	97	—
8	Sand	0	—	0	68	22
	Loamy sand	0	—	1	92	—
	Medium loam	0	—	0	93	—
	Clay	0	—	0	92	—

* Expressed as no. per 100 sclerotia. At each depth, 36 sclerotia were planted in each soil type.

Few sclerotia were thus infected, and the failure is again attributed to filtration of spores from suspension and their accumulation in the superficial soil layers. Infection of apothecia indicated the presence of viable spores in these layers. The loamy sand used in this experiment was very similar to that in the pot trial described above. An important difference in procedure was that a pronged planting device was used in the pots, which led to the soil above the sclerotia being less tightly packed than if the soil had been settled by tapping the containers down on a hard surface, as was done with the pipes. The former conditions were therefore exceptionally favourable to spore penetration. Owing to these indications of spore filtration, future experimental inoculations were made with solid inocula mechanically mixed with the soil.

With cornmeal-sand inoculum

The inoculum was prepared by growing *C. minitans* in 3 % cornmeal-sand in flasks for 3 months. The material was shaken out on to newspaper and dried at laboratory temperatures immediately before use. A powdery inoculum was thus produced.

The factors varied in this experiment were method of inoculation, depth of burial of sclerotia, and soil type. The inoculum was applied in two ways: (1) evenly to the surface of the soils and lightly raked in, to about 0·25 in. depth after planting the sclerotia; (2) by mixing 1 % with the soils before planting with sclerotia. In (1) sufficient inoculum was applied just to cover the surface; this was 25 % of that used in (2). Sclerotia, obtained from carrot culture, were planted at three depths, 0·5, 1, and 2 in., in the course of filling the containers with soil. The soils used were the light loamy sand and the heavy Gault clay from the University Farm.

The experiment was done in two parts. One lot of sclerotia was planted in rows 1 in. apart in seed-boxes, which were let into the surface of a field at the Farm to approximate to field conditions. The second lot was buried in pots, which were kept on the roof of the School of Agriculture. Summer conditions prevailed, from July to early October 1954, and the moisture content of the potted soil was allowed to fluctuate between 30 and 60 % M.H.C. The sclerotia in the pots were dug out after 9 weeks and those in the boxes after 10–11 weeks, and examined visually for *C. minitans*, with confirmation where necessary by the water test.* From the pots, virtually 100 % of the sclerotia were recovered, and from the boxes some 90 %. Since infection for 11 weeks never totally destroys a sclerotium, those not recovered were just 'missing' and were not necessarily infected.

Table 4 shows that a high degree of infection was given by the preparation mixed in as a 1 % amendment in both soil types and at all depths. Where the preparation was applied to the surface there was a much lower degree of infection, of similar order in both soils, and an indication of more infection at the half-inch depth. There was good agreement between results in boxes and those in pots.

The boxes were left down for 2 years. In May 1955, 8 months after harvesting the sclerotia, the soils to which 1 % inoculum had been added and the uninoculated control soils were tested for *C. minitans* by two methods. First, samples of soil were taken from three depths, as above, and plated on to cultures of *S. trifoliorum*. Sterile glass tubing was pushed into the soil and a tiny piece from the lower end of the core used to inoculate the cultures. Secondly, sclerotia were placed into the holes so formed. They were dug up in July 1955, after 10–11 weeks burial. In September 1956, after a further period of 14 months, the boxes were weeded and sclerotia planted in layers at the three depths as before. They were left for 14–16 weeks and dug up in December 1956. There was no influence of depth on persistence of *C. minitans*, and the results are sum-

* An observation on production of apothecial stipes may be appropriate here. Half of the sclerotia used in the box trial were obtained from carrot cultures set up 8 months previously and half were from 3-month-old cultures. There was no difference in their susceptibility to *C. minitans*, but a great difference in production of apothecial stipes was noted on examination in early October 1954. In clay soil, of 165 older sclerotia recovered uninfected from the boxes (including those from the controls), 84 were producing 201 stipes. Of 170 younger sclerotia, 4 were producing 6 stipes. In loamy sand, of 77 older sclerotia recovered uninfected, 56 were producing 145 stipes; of 79 younger ones, 3 produced 3 stipes. (The over-all numbers recorded from loamy sand are lower, since some of each age had been dug out and mixed before the difference in behaviour was noticed.)

Table 4. *Parasitism of* Sclerotinia trifoliorum *sclerotia in soils after* 9–11 *weeks by cornmeal-sand cultures of* Coniothyrium minitans

Soil type	Application of inoculum	Depth (in.)	Percentage sclerotia infected 0·5	1·0	2·0	Mean
Loamy sand	To soil surface	Box	29	10	9	16
		Pot	30	0	0	10
	Mixed with soil	Box	94	84	94	91
		Pot	87	97	100	95
	Uninoculated control	Box	5	17	10	11
		Pot	0	0	0	0
Gault clay	To soil surface	Box	19	3	3	8
		Pot	60	7	40	36
	Mixed with soil	Box	74	87	95	85
		Pot	100	97	100	99
	Uninoculated control	Box	0	0	0	0
		Pot	10	0	0	3

Each percentage figure is based on *c.* 35 sclerotia in the box replicate (i.e. those recovered from a maximum of 40) and on 30 sclerotia from each pot.

Table 5. *Persistence of* Coniothyrium minitans *in soils*

Soil type	Treatment in July 1954	Sclerotia* infected (%) Sept. 1954	Infective soil samples (%) May 1955	Sclerotia† infected (%) July 1955	Sclerotia‡ infected (%) Dec. 1956
Loamy sand	Cornmeal-sand inoculum mixed with soil	91	86	59	89
	Uninoculated control	11	13	43	40
Gault clay	Cornmeal-sand inoculum mixed with soil	85	96	91	83
	Uninoculated control	0	33	22	11

* From Table 4.
† Based on minimum of 22 recovered sclerotia.
‡ Based on minimum of 60 recovered sclerotia.

marized in Table 5. It was also noted that no apothecia were produced by this batch of sclerotia.

C. minitans was shown to persist in both soils for at least 14 months, during which period the soils were not disturbed and weeds grew unchecked. It is probable that it persisted 2 years from the initial incorporation in 1954, as it is unlikely that spores produced on the 1955 sclerotia, most of which were dug out, would be responsible for much infection.

A problem was presented by the spread of *C. minitans* to the uninoculated control boxes. In the Gault clay, for example, no infection was recorded on sclerotia in 1954, but by May 1955 the soil was appreciably contaminated. Since the boxes were adjacent, and were separated by only a few inches of field soil it is possible that there was slight transference of inoculated soil either by accident during the removal of the first batch of sclerotia in 1954, or subsequently by wind and rain or by soil fauna. Nevertheless, this explanation conflicts with the difficulty of spread of spore suspensions through the soils. It may be that individual spores

distributed in suspension become readily bound to soil crumbs, but that small masses of spores may be dispersed by soil water or by soil fauna.

C. minitans is not a soil inhabitant in the same sense as are, for example, *Trichoderma viride* or *Gliocladium roseum*, both of which commonly appear when soil particles are placed on cultures of *S. trifoliorum*. *C. minitans* was not found in three samples of loamy sand from the University Farm or from a garden loam, whereas both the other fungi occurred consistently in these as well as in soil from the box inoculated with cornmeal-sand, as is shown in Table 6.

Table 6. *Isolation of certain fungi on cultures of* Sclerotinia trifoliorum

		Occurrence of fungi in 20 platings		
Soil type	Source	*C. minitans*	*T. viride*	*G. roseum*
Loamy sand	Cornmeal-sand inoculum (mixed with soil from Farm Field)	19	8	13
	Farm Field (1)	0	13	7
	Farm Field (2)	0	9	13
	Gravel Pit	0	7	12
Garden loam	Wimbledon	0	6	13

Both *T. viride* and *G. roseum* can overrun *S. trifoliorum* on potato-dextrose agar, but neither was able to infect sclerotia in soil. Two treatments with these fungi had been included in the pot experiment detailed in Table 2, at spore concentration 'S' and with sclerotia buried at 1 in., without positive results.

Discussion

This work has shown that under certain conditions *C. minitans* is an effective pathogen against sclerotia of *S. trifoliorum*. Only the lowest seasonal temperatures prevented its development; normal autumn or spring temperatures were found quite adequate for infection. For good control it was necessary to mix the inoculum intimately with the soil, probably so that direct contact was established between spores and sclerotia. Certain points may be considered in relation to possible use of *C. minitans* in biological control of *S. trifoliorum*. Probably the best time to attack sclerotia in the field would be during the preparation of the seed-bed of the crop to be protected. At that time a *C. minitans* preparation could be thoroughly worked into the soil and, since the treatment of the land preceding the seed-bed would be the last deep cultivation for that crop, any sclerotia present below the critical depth would not be able to produce apothecia. Sclerotia must be killed before apothecia appear, and a preparation applied either to spring or autumn seed-beds would have ample time to destroy sclerotia before the following autumn. It may thus be possible to shorten the rotation period between successive red clover crops, officially recommended as 8 years where the disease is troublesome (Min. Agric. & Fish. Adv. Leaf. No. 266). It must be noted that good control is so far of the order of 90 % kill. It has yet to be found how com-

plete the elimination of sclerotia must be to control clover rot. Nothing is known of the density of ascospores required to cause appreciable infection under favourable conditions. There is some evidence that the source of infection of a newly sown clover crop may be ascospores produced in another field. For example, a field in West Suffolk, where clover was grown on land which had borne currant bushes for 15 years, was yet fairly heavily attacked. Unless the sclerotia had survived this period, the ascospores must have come from elsewhere. If this is found to be general, a systematic inoculation of fields known to contain sclerotia may be envisaged. *C. minitans* persists in soils for at least one year and probably for two, and further work may show a longer persistence. It may be found that treatments are relatively permanent.

It is not known where or even whether *C. minitans* lives as a saprophyte in nature. The origin of natural infections of sclerotia is obscure; they may come from pre-existing infected sclerotia, from the soil or from another habitat such as stems or twigs. Spores certainly persist ungerminated in disintegrated sclerotia for at least a year. Species of *Coniothyrium* occur as minor plant pathogens, and are sometimes found in lists of soil fungi, but so far *C. minitans* has only been found in connexion with sclerotia. Further information on its ecology would be of great interest.

It is a pleasure to thank Prof. Sir Frank Engledow, F.R.S., for his stimulating interest throughout the course of this work. I wish to express my thanks for the help rendered by the National Agricultural Advisory Service during the studies on distribution of *C. minitans*; to Mr E. C. Large and Dr F. Joan Moore for information and assistance in connexion with the Ministry of Agriculture Clover Rot Survey, to the late Dr W. A. R. Dillon Weston and to Miss June Ives of Cambridge, Mr J. T. Marshall of Bury St Edmunds and Mr A. L. Boyd of Bedford for much personal help and for arranging visits to infected fields, and to many officers for sending me samples of sclerotia.

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STUDIES ON LARCH CANKER

II. THE INCIDENCE AND ANATOMY OF CANKERS PRODUCED EXPERIMENTALLY EITHER BY INOCULATION OR BY FREEZING

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(With Plates 24–27)

The anatomy of cankers produced in European larch by inoculation with *Trichoscyphella willkommii* in a frost-free locality proved to be identical with that of naturally occurring cankers, except for the absence of a frost ring in the wood. An apparatus was devised, by means of which limited areas of the stems of potted larch trees could be subjected to freezing of a known intensity. Cankers resulting from this treatment were also identical anatomically with naturally occurring cankers, except that the frost ring was incomplete and fungus mycelium absent. Trees of European larch susceptible to canker in the field produced a significantly greater number of cankers when inoculated with *T. willkommii* than did trees resistant in the field.

These results are discussed, and it is concluded that *T. willkommii* as well as frost, is involved in the production of cankers in European larch in the field.

Introduction

The anatomy of cankered larch stems is now well known, having been studied by a number of workers during the past century. Willkomm (1867–8) and Hartig (1880) described the anatomy of typical cankers, but Hiley (1919) was the first to study their developmental anatomy. He found that, in a young canker, a small area of dead phloem and cambium, permeated by the mycelium of the fungus now known as *Trichoscyphella willkommii* (Hart.) Nannf. was present. The following spring, the cambium on either side of the dead area cut off first parenchymatous cells and later abnormal tracheids, before producing normal wood. Resin ducts and tannin cells also occurred. The following winter a further area of cambium would be killed, and during the subsequent spring and summer further abnormal tissues would be produced on either side of the now enlarged canker. This process could continue indefinitely.

Day (1931) showed that the initiation of a canker frequently coincided with the production of a frost ring. He concluded that if frost damage were severe enough to kill the cambium locally, then a canker would result. He pointed out that the types of abnormal cells and tissues associated with frost rings were similar to those associated with cankers. These observations were extended by Day & Peace (1934), who were able to produce cankers experimentally, though only with difficulty, by artificially freezing young trees. Such cankers were identical anatomically with those occurring naturally. Hahn & Ayers (1943) were able to produce cankers

by artificial inoculation with *T. willkommii* in an area where frosts did not occur; they state that abnormal tissues were present around the cankers, but give no details. Latour (1949) listed the anatomical abnormalities which could, in his opinion, be taken as diagnostic of frost damage. He found all these abnormalities in naturally occurring cankers and concluded that frost must therefore play an important part in their formation.

The anatomy of cankers produced in the absence of frost does not appear to have been studied in detail, and the main object of the present investigation was to carry out such a study and to compare the anatomy of cankers produced by inoculation with those of frost-induced or naturally occurring cankers.

Cankers produced by inoculation with *Trichoscyphella willkommii*

Methods

A number of cankers produced by inoculation with *T. willkommii* were obtained from two series of experiments in a frost-free locality near Penzance. Details concerning the inoculation techniques employed, and the trees and cultures used in the first series (trees inoculated on 18 April 1951) have already been reported (Manners, 1953). The trees used in the second series, inoculated on 10 January 1952, came from the same planting as those employed in the first series. Half of them were of a Scottish provenance, the other half of an Alpine provenance, it being well established (Forestry Commission, 1948) that trees of Alpine provenance are more susceptible in the field than those of Scottish provenance. Most of the cultures inoculated were those used previously, but 15/1 replaced 15/5 (both were derived from material collected in the New Forest on 23 March 1950).

Both series of experiments ended on 23 July 1953, when the inoculated portions of the stems were cut out and preserved in 70% alcohol with 5% formalin and 2% glycerol. Subsequently, transverse sections were cut through the site of each inoculation with a Reichert sledge microtome. Stems of up to 4 cm. diam. could be cut entire, but it was necessary to split larger stems and re-assemble the parts after sectioning. All sections were cut at 20μ and stained by the method described by Cartwright (1929). The staining times given by him were modified, the sections being left for 5 min. in the safranin and for 45 sec. in the hot picro-aniline blue.

The severity of attack was assessed both in the field immediately before harvesting and in the laboratory after examination of the sections. The field results were assessed according to the following scale, modified from that described previously (Manners, 1953):

0 Wound healing normally.
1 Wound healing slowly, sometimes with slight necrosis.
2 Small canker, usually without apothecia.
3 Large canker, usually with incipient apothecia.
4 As 3, but in addition, the stem or branch beyond the point of inoculation is dead or dying.

For the anatomical assessment, the following scale was employed:

0 Wound healed normally (always within the growing season of inoculation).
1 Wound healed within season of inoculation, but rather slowly.
2 Wound healed or very nearly so in season of inoculation, but further damage to cambium in following year.
3 Wound healed in season of inoculation, but considerable damage to cambium the following year, resulting in the formation of a canker.
4 Wound not healed in year of inoculation. Further damage to cambium in following year resulting in the formation of a canker.

The influence of provenance on susceptibility

The results of both field work and anatomical assessments of the 1951 and 1952 Penzance experiments are given in Tables 1 and 2. It was not

Table 1. *Inoculations at Penzance*, 1951

T. willkommii	No. of trees inoculated	Field assessment		Anatomical assessment	
		Scottish	Alpine	Scottish	Alpine
Culture no.					
15/5	4	2 0	3 3	2 –	2 3
18/1	4	0 2	2 0	– 2	3 –
20/1	4	3 2	3 4	3 4	4 –
103/3	4	3 0	4+ 1	3 –	– –
Control: sterile agar	8	0 0 0 0	0 0 0 0	– – – –	– – – 0

Table 2. *Inoculations at Penzance*, 1952

T. willkommii	No. of trees inoculated	Field assessment		Anatomical assessment	
		Scottish	Alpine	Scottish	Alpine
Culture no.					
15/1	4	0 0	0 0	— —	0 0
18/1	4	2 3	4 3	3 3	4 —
20/1	4	2 0	2 3	1 0	2 3
103/3	4	0 1	4 3	0 1	3 3
Control: sterile agar	4	0 0	0 0	— —	0 —

possible to examine all trees anatomically: some, mostly trees in which the inoculation wound had apparently healed normally, were omitted. The anatomical and field results agree well, indicating that the latter may reasonably be employed as an index of the severity of the attack and of the extent of the damage to the cambium. The field results for the 2 years (taken together and omitting cultures only used in 1 year) were examined statistically. The data being of the nature of 'scores', an analysis of variance assuming that the distribution is normal can only give a suggestion as to what differences may be significant. Such an analysis was, however, performed and the results indicated that the trees of Alpine provenance were more susceptible than those of Scottish provenance (difference significant at the $P = 0{\cdot}05$ level). There was also a suggestion (difference scarcely significant at the $P = 0{\cdot}05$ level) that the relative

virulence of the cultures used altered from one year to the other. This cannot easily be explained and requires further investigation. Further experiments on the relative susceptibility to *T. willkommii* of various provenances of European larch are in progress, but the present results, although somewhat preliminary in nature, indicate that there is a correlation between susceptibility in the field and susceptibility to the fungus.

The anatomy of cankers produced by inoculation

In order to illustrate the mode of development of cankers produced by inoculation, a few examples will be considered in detail.

(1) *Severe attack* (tree 104, Alpine provenance, field assessment grade 3+, anatomical assessment grade 4). Field record:

18. iv. 51. Inoculated with *T. willkommii* (culture 20/1) on a 2-year-old portion of the main stem.
5. ix. 51. Wound more or less healed.
10. i. 52. Wound more or less healed but incipient apothecia appearing round it.
2. iv. 52. Wound more or less healed, but with a sunken area around a central mass of resin. Numerous incipient apothecia present.
2. ix. 52. Large canker, tree growth poor.
23. vii. 53. Large canker (no apothecia). Tree growth very poor: tree harvested.

A transverse section through the canker is illustrated in Pl. 1, fig. 1. The damage to the cambium in 1951 may be due partly to the effect of wounding, but by the end of the 1951 growing season, the wound had very nearly healed (healing was complete in the plane of section illustrated). A large additional area of cambium was, however, killed during the winter of 1951–2, and growth during the summer of 1952 did not overgrow the canker to any extent. There was further damage to the cambium during the winter of 1952–3, so that the tree was nearly ringed at the time of harvesting.

(2) *Moderate attack* (tree 113, Alpine provenance, field assessment grade 3, anatomical assessment grade 3). Field record:

18. iv. 51. Inoculated with *T. willkommii* (culture 15/5) on a 2-year-old portion of the main stem.
5. ix. 51. Wound resinous, partly healed.
10. i. 52. Wound healed.
2. iv. 52. Wound healed, but incipient apothecia appearing round it.
2. ix. 52. Wound open, cankered. No apothecia visible.
23. vii. 53. Large canker with incipient apothecia. Tree harvested.

A transverse section through this canker is illustrated in Pl. 1, fig. 2. In this tree serial sections confirmed that the wound completely healed during 1951. By the beginning of the 1952 growing season, however, a large segment of cambium had been killed, presumably by mycelium which had persisted in the bark. This dead area was partly overgrown during the 1952 growing season, but during the following winter further areas of cambium were killed and the canker enlarged further.

The fate of the fungus during the autumn and winter in cases where the original inoculation wound heals completely, as far as cambium and xylem are concerned, requires further investigation. Presumably it persists in the moribund phloem or cortex over the canker, growing inward to re-invade the cambium during the tree's period of dormancy.

(3) *Control inoculation* (tree 134, Alpine provenance, field and anatomical assessment, grade 0). As on all the other control trees the wound healed within the season of inoculation (Pl. 2, fig. 2). This tree was inoculated on 10 January 1952 and had healed by 2 April 1952. This healing was permanent, there being no recurrence of damage to the cambium.

The investigations of Day (1931) and Latour (1949) have given a detailed picture of the pathological anatomy of naturally occurring cankers. The cankers produced by inoculation were examined in detail and their anatomy compared with that of the naturally occurring cankers. Cankers were chosen in which the inoculation wound had healed (anatomical assessment grade 3), so that abnormalities resulting from the wounding could be ruled out. The canker produced on tree 131, a larch of Alpine origin inoculated on 10 January 1952, may be taken as an example (Pl. 2, fig. 1). The inoculation wound had healed by the end of the 1952 growing season. During the winter of 1952–3, however, nearly half the circumference of the cambium was killed and abnormal tissue developed at the margins of the dead cambium. The types of abnormality included:

(1) A lignified parenchyma with simple pits (Pl. 3, fig. 1).

(2) Immediately outside this, irregularly shaped tracheids with numerous bordered pits on the horizontal walls (Pl. 2, fig. 3).

(3) Outside this again, tracheids elongated tangentially, narrow and oblong in transverse section ('coffin cells') (Pl. 2, fig. 3).

(4) Enlarged and bent medullary rays (Pl. 3, fig. 1).

There was nothing resembling a frost ring in these cankers, but irregular parenchymatous cells, filled with tannin or resin, extended into the wood for a short distance on either side of the canker, just after the beginning of the 1953 wood (Pl. 3, fig. 1). This suggests that the time of maximum activity of the fungus might be in early spring.

The details of the anatomy of control trees were also studied. In all cases the inoculation wound had healed rapidly, but during the early stages of healing all the abnormal cell types described above were seen (Pl. 2, fig. 4).

Cankers produced by local freezing

Methods

The experimental production of cankers by the use of low temperatures is not an easy matter. Day & Peace (1934) were able to produce a small number of cankers artificially in potted trees of European larch by subjecting them to low temperatures in a refrigerator, but the proportion of trees producing cankers was very low. Day (1931) showed that, in mature trees, certain specific parts of the cambium sprang into activity earlier in the spring than the rest of the cambium. Such areas would be especially liable to frost damage, which would take the form of canker

formation. In the small trees necessarily employed in freezing experiments the whole cambium becomes active more or less simultaneously, so low temperatures cause die-back, rather than cankers. Hence it seemed that a method involving local freezing would be desirable. The local application of ice or solid carbon dioxide proved difficult to control so an apparatus was constructed whereby a stream of air at a known low temperature could be directed against a given area of bark. (The apparatus is illustrated in Pl. 3, fig. 2.) Air is pumped into the apparatus by means of a blower, passed through a constant pressure flow regulator, dried over calcium chloride (to avoid condensation) and passed through a 40 ft. coil of copper tubing immersed in acetone saturated with solid carbon dioxide (giving a temperature of $-60°$C.) contained in a large Dewar vessel. The cooled air passes into a moulded polyvinyl resin head, constructed in such a way that the stem of a potted larch can be held securely while a jet of cold air is directed on to one side of the stem. A type 'F' 'thermistor' is inserted into the head in such a way that the air temperature at the point where the air hits the bark is measured. The 'thermistor' is connected to a resistance thermometer circuit employing a galvanometer sufficiently sensitive to allow the temperature to be read to the nearest $0{\cdot}1°$C. Several heads are employed, with different sleeve sizes to accommodate trees of differing stem diameter. The potted plants are suspended by means of a block and tackle in such a position that the appropriate part of the stem can be inserted in the sleeve without placing any strain on it. By adjusting the head of water in the constant pressure flow regulator the temperature of the air stream at the bark surface can be kept constant within $0{\cdot}25°$C. at any level between 0 and $-15°$C. for several hours.

The trees employed in these experiments were maintained both before and after treatment in frost-proof cold frames (thermograph records confirmed that in no case were the trees accidentally subjected to frost). Portions of stem 3–4 years old, with few or no laterals, were selected for freezing. The trees were harvested 2–3 years after treatment, and the frozen areas sectioned and stained as described above for inoculated trees.

The anatomy of cankers produced by local freezing

Between 1952 and 1954 a number of preliminary experiments were carried out to determine the appropriate duration and intensity of freezing for canker production. It was found that freezing for 2 hr. at $-10°$C. (3- to 4-year-old stems) or $-5°$C. (2-year-old stems) produced the required result. In 1954, 1955 and 1956 large-scale freezing experiments were carried out, using these experimental conditions. These experiments are still in progress and will be reported in full later, but the salient features of cankers caused by local freezing have been studied in certain trees from those experiments already harvested.

Tree 328 (Forestry Commission provenance no. 50/252), a tree of an alpine strain of European larch, produced a typical canker. The tree was frozen on 10 May 1955; an obvious canker had developed by 30 August 1955, and this never healed over. The general appearance of the canker was similar to that of cankers caused by *T. willkommii* except that there

was very little resin production. Consequently, the dead bark over the cankered area peeled off, revealing the wood. The tree was harvested on 20 September 1956 and the canker sectioned (Pl. 4, fig. 1).

Anatomically the canker was similar to those caused by the fungus, except that mycelium was, naturally, absent. This canker did not heal within the year of freezing, but when frost-induced cankers did heal within a single growing season, then healing was always permanent. This is illustrated by tree 360 (Forestry Commission provenance no. 50/265), a tree of a Sudeten strain of European larch (Pl. 4, fig. 2), which was frozen for 2 hr. at $-10°$C. on 28 September 1954 and harvested on 21 May 1955. The anatomical abnormalities characteristic of fungus-induced cankers were also present in those induced by freezing. They included simple pits, bordered pits on the horizontal walls of deformed tracheids, 'coffin cells' and distorted and swollen medullary rays. No complete frost ring was present, but, in contrast to the fungus-induced cankers, these cankers often had a partial frost ring, extending approximately half way round the stem.

Discussion

The anatomical differences between naturally occurring cankers, those produced by artificial inoculation with *T. willkommii* and those resulting from local freezing are slight, the most important being the lack of resin in frost-induced cankers. Various forms of abnormal xylem tissue associated with cankers, such as parenchyma with simple pits, distorted tracheids with bordered pits on their horizontal walls, 'coffin cells' and bent and swollen medullary rays were attributed by Day (1931) and more particularly by Latour (1949) to frost damage. All these abnormalities, however, occur in cankers produced by fungal inoculation just as much as in cankers produced by local freezing or in naturally occurring cankers. They also occurred in the wound tissue formed during the healing of the slits made in control inoculations, and they clearly represent a general reaction to damage to the cambium. The bearing of these findings on theories of canker causation in nature merits consideration. The invariable presence of the frost ring in connexion with the initiation of naturally occurring cankers (Day, 1931) indicates that frost is involved, while the abundant resin production, the failure of the cambium to heal and the invariable presence of a fungus known to be a wound parasite indicate that the fungus, too, is involved.

Day & Peace (1934), in a comparative study of several strains of European larch, showed that there was a connexion between susceptibility to canker in the field and susceptibility to frost, and a correlation between field susceptibility and susceptibility to *T. willkommii* has emerged during the present investigation. Since field susceptibility is correlated with both susceptibility to frost and susceptibility to *T. willkommii*, there must be a correlation between susceptibility to each of these two agents. The possible implications of such a correlation merit consideration. One possible explanation is that strains of European larch resistant to canker in the field have been unconsciously selected over a period of many years for

resistance to both frost and fungus. Another possibility is that a single genetical or biochemical factor governs resistance to both agents, or that two closely linked biochemical or genetical factors are involved. Other types of causal relationship between the two types of susceptibility may be postulated. It is inconceivable that the presence of the fungus could influence the occurrence of frost, but the presence of the fungus within the tissues might increase their susceptibility to frost; frost damage might easily increase the susceptibility of the tree to fungal attack. In either case the presence of both frost and fungus might be necessary for canker formation to occur in the field.

The above considerations provide support for the theory of canker formation put forward by Langner (1936). He suggested that the fungus entered through frost cracks and established itself in tissues rendered dead or moribund by the action of frost. He also postulated that the persistence of cankers was a cyclical process: evidence was obtained indicating that the fungus produced toxins rendering cells adjoining the canker especially liable to frost damage. Only a slight frost would be required to kill such cells, which would then be invaded by the fungus. A continuation of the whole process would lead to progressive enlargement of the canker. This theory, implying as it does a close interdependence between the action of frost and that of the fungus, provides an explanation for the observed correlations between field susceptibility and susceptibility to frost on the one hand and the fungus on the other. Acceptance of Langner's theory implies that the fungus can probably discharge its ascospores at low temperatures, since the period for which frost cracks are open to fungal invasion is probably limited, and that the mycelium from germinating ascospores can enter frost cracks and invade frost-damaged tissues. Experiments designed to determine whether these implications are well founded are in progress.

If it is assumed that *T. willkommii*, as well as frost, is a prerequisite for the formation of cankers in nature, the inoculum of this fungus must be present for canker formation to occur. This aspect of the problem has been ignored by most recent authors, perhaps owing to the frequent failure to distinguish between *T. willkommii* and the closely related saprophyte *T. hahniana* (Seaver) Manners. The relationship between the incidence of cankers in young plantations, the number of spores of *T. willkommii* present in the air, and the proximity of cankered stands of larch, might well repay investigation.

I wish to express my thanks to Mr T. R. Peace and other Forestry Commission Officers for their kindness in supplying material and information, to Mr W. E. Middleton for technical assistance and to Mr F. A. Barrett and to the Forest Products Research Laboratory for photography. This work was carried out with the aid of a grant from the Forestry Commission, to whom acknowledgement is made.

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EXPLANATION OF PLATES 24–27

Plate 24

Fig. 1. Tree 104 (Penzance). T.S. through canker caused by inoculation with *Trichoscyphella willkommii* (anatomical assessment, grade 4). × 8.

Fig. 2. Tree 113 (Penzance). T.S. through canker caused by inoculation with *T. willkommii* (anatomical assessment, grade 3). × 5.

Plate 25

Fig. 1. Tree 131 (Penzance). T.S. through canker caused by inoculation with *T. willkommii* (anatomical assessment, grade 3). × 6.

Fig. 2. Tree 134 (Penzance). T.S. through wound in control tree (anatomical assessment, grade 0). × 3·5.

Fig. 3. Tree 131 (Penzance). Abnormal tracheids with pits on their horizontal walls. × 400.

Fig. 4. Tree 140 (Penzance). Wound tissue at site of control inoculation. × 400.

Plate 26

Fig. 1. Tree 131 (Penzance). Bent and swollen medullary rays, and lignified resin-soaked parenchyma with simple pits, at edge of canker. × 90.

Fig. 2. Apparatus employed for the local freezing of potted larch trees. The flask containing the cooling coil is immediately behind the tree.

Plate 27

Fig. 1. Tree 328 (Southampton). Canker resulting from local freezing at −10° C. for 2 hr. × 7·5.

Fig. 2. Tree 360 (Southampton). Damage resulting from local freezing at −10° C. for 2 hr. × 10.

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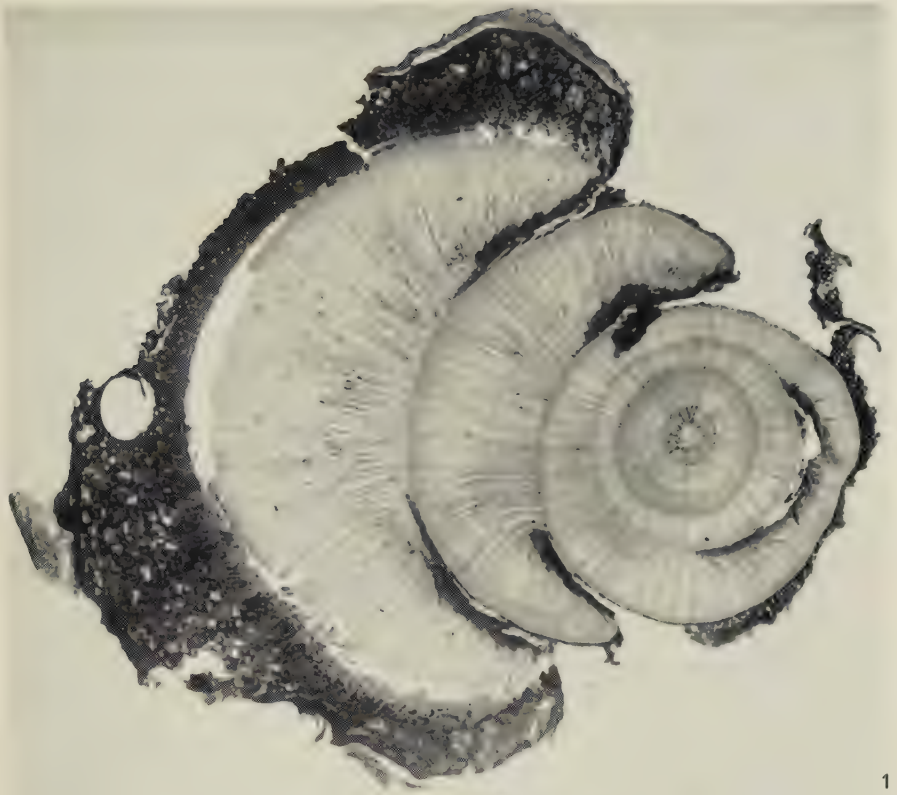

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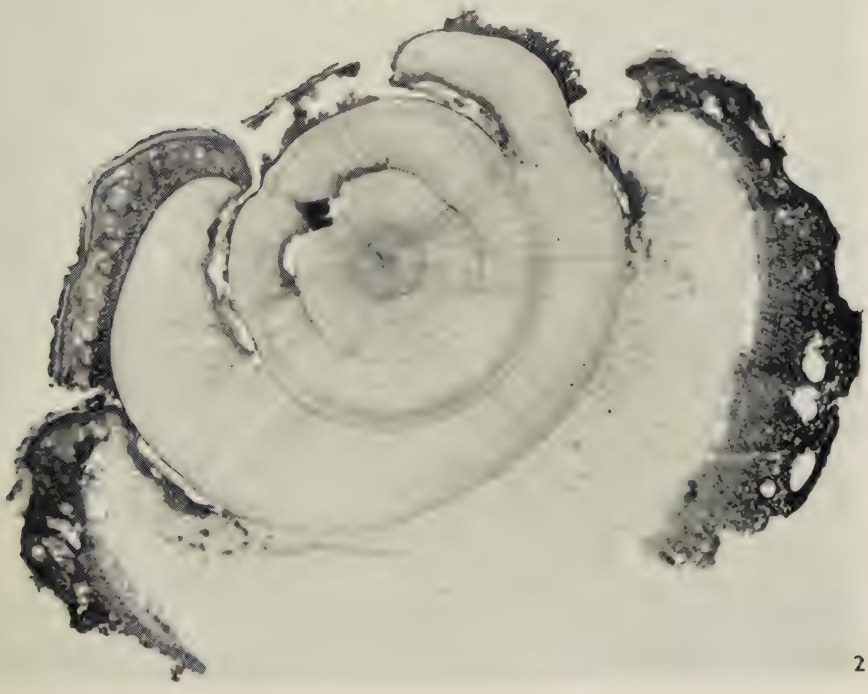

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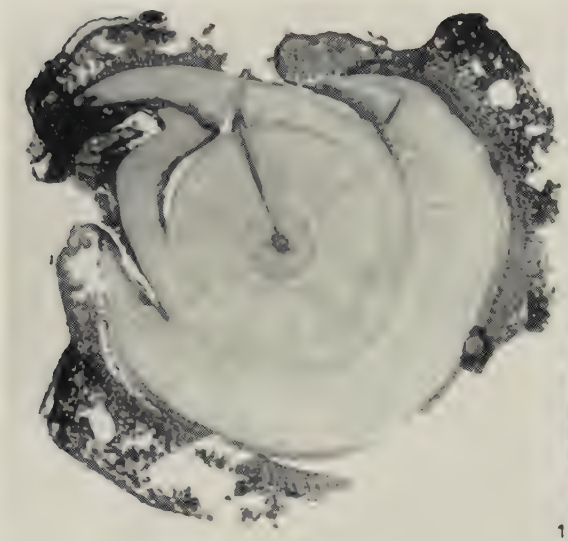

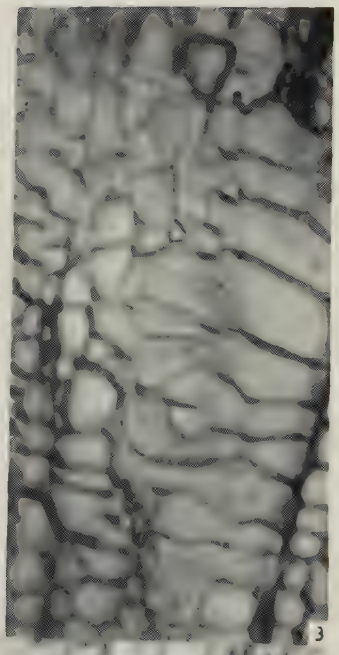

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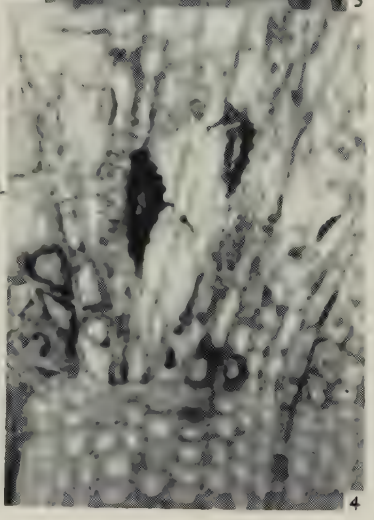

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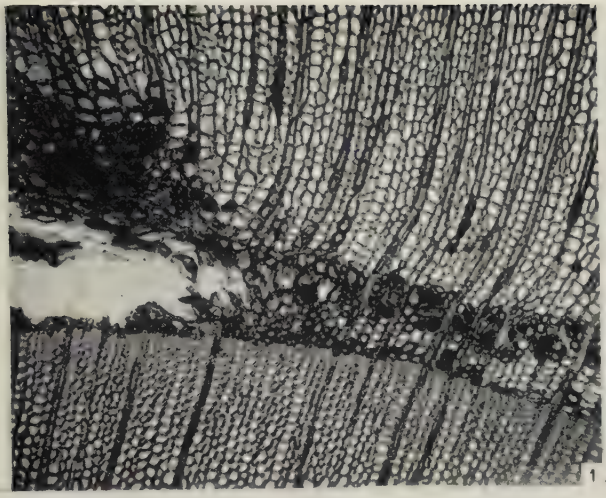
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Trans. Brit. mycol. Soc. **40**, (4), 509–522 (1957).

GRAMINICOLOUS PYRENOMYCETES

VI. CONIDIA OF *OPHIOBOLUS HERPOTRICHUS*, *LEPTOSPHAERIA LUCTUOSA*, *L. FUCKELII*, *L. PONTIFORMIS* AND *L. EUSTOMOIDES*

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(With 6 Text-figures)

Cultural studies have shown that:

Ophiobolus herpotrichus forms brown transversely septate pycnospores with a terminal bristle-like appendage, possibly belonging to the form-genus *Urohendersoniella*.

Leptosphaeria luctuosa and *L. fuckelii* form pycnospores of the *Phaeoseptoria* type.

L. pontiformis and *L. eustomoides* form pycnospores of the *Hendersonia* type.

Ophiobolus herpotrichus (Fr.) Sacc., *Sylloge Fungorum*, **2**, 352

Perithecia of this fungus are common on basal internodes of many grasses from March to July. We have collected it on the following hosts: *Agropyron repens*, *Arrhenatherum elatius*, *Bromus sterilis*, *Festuca ovina*, *Holcus mollis*, *H. lanatus*, *Zerna ramosa* and on wheat and barley stubble. The following description is based on a specimen on *Agropyron repens*.

Perithecia: scattered or in groups on the stem or leaf sheath, immersed, with the neck penetrating; spherical or flattened at the base, black, surrounded by numerous dark brown, thick-walled hairs about 4 μ wide; 250–500 μ diam., neck long or short, up to 150 μ long. *Asci*: numerous, bitunicate, cylindrical or club-shaped, tapering to a long stalk below; 8-spored, 110–218 × 8–11 μ; surrounded by numerous filiform, branched paraphyses. *Ascospores*: filiform, yellow to pale brown, with numerous small guttules and up to twenty septa when mature, nearly as long as the asci, 120–185 × 2–3 μ.

Fries (1823) cites Scler. Suec. No. 52 as an exsiccatum of his *Sphaeria herpotricha*. Although a fragment of this specimen is preserved in the Berkeley collection at Herb. Kew., we have failed to find material of *Ophiobolus* on it. Our specimens have, however, been matched with Desmazières, Crypt. France, Ser. 1, 1283 from Herb. Kew. Desmazières on the Herbarium packet claims to have seen Fries's exsiccatum 52.

Cultures. Cultures were prepared from single ascospores and asci from numerous collections. All isolates on oat agar produce dense white colonies at first; later the aerial mycelium becomes smoky grey, olive green or black and a characteristic citrine colour is formed in the medium. Pycnidia were found after 6–10 weeks. The pycnidia are similar in form to perithecia, with or without necks, black, globose, 200–550 μ diam. Golden-brown masses of spores exude from the necks. The pycnospores have a cylindrical

body, truncate at the base, tapering above to a pointed bristle-like appendage, and are 5–6 septate with one to several guttules per cell; 71–104 × 3–4 μ.

Pycnidia on the host. Similar pycnidia have been collected on *Agropyron repens* associated with perithecia, from which they are difficult to distin-

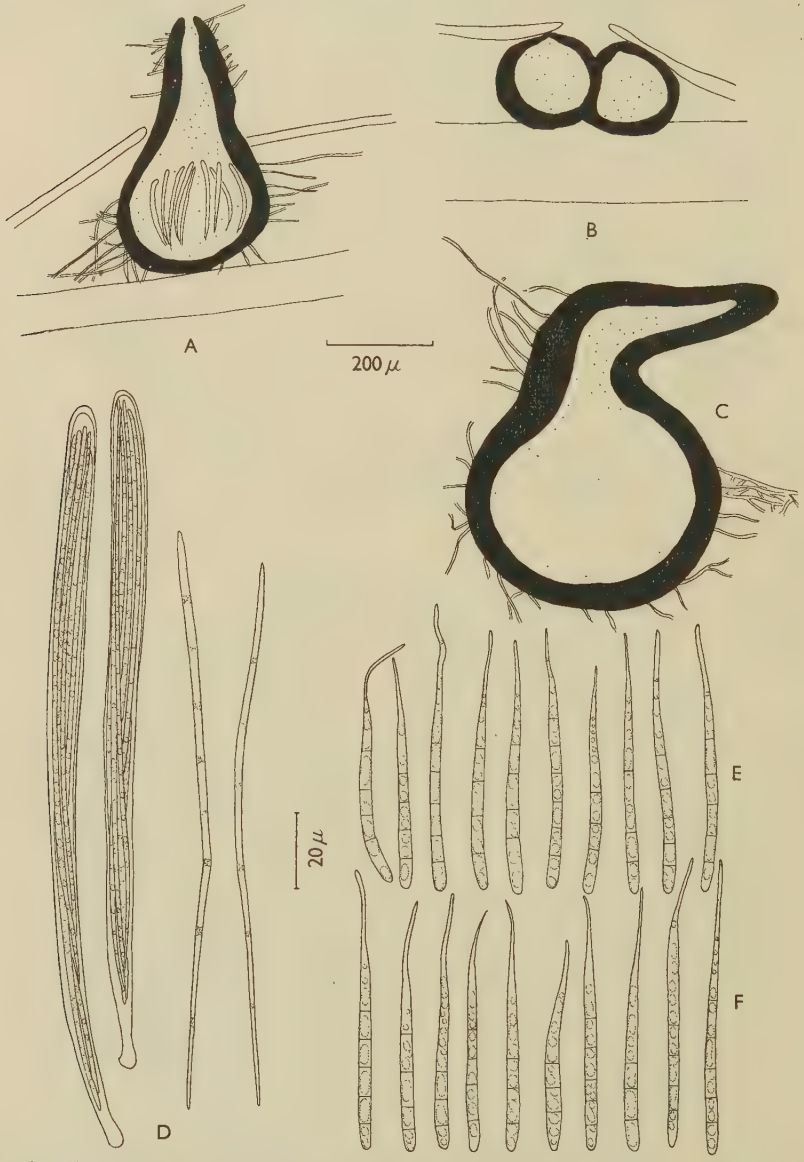

Fig. 1. *Ophiobolus herpotrichus* (Herbarium numbers in parentheses). A, L.S. wheat stem and perithecium (1763); B, L.S. *Agropyron* stem and pycnidia (1762); C, section of oat agar culture derived from a single ascospore showing a pycnidium (1758); D, asci and ascospores (1763); E, pycnospores from *Agropyron* (1762); F, pycnospores from a single ascospore culture on oat agar (1758).

guish. They have been collected from July to September on the withering lower leaves of *Agropyron* culms, and also on the stems. Pycnidia on *Agropyron* were 200–550 μ diam. and contained pycnospores resembling closely those described above, measuring 69–116 × 3–4 μ. Isolates from such pycnospores resembled those derived from ascospores, forming pycnospores within 2 months.

The dimensions of other collections and cultures derived from them are given in Table 1.

Guyot (1925) has reviewed earlier records claiming conidial connexions with *Ophiobolus herpotrichus*, but none is based on culture work. Only Tulasne & Tulasne (1863) seem to have observed pycnidia resembling our cultures, but they did not clearly ascribe these to *O. herpotrichus*. They describe 'stylospores, which are linear-lanceolate, curved, acute at both ends, 6–8–10 locular...; when mature the seeds are dark-coloured and measure 25–35 μ; but besides the genuine pycnidia, there at times occur some with stylospores nearly twice as long, with more septa, clavate and attenuated downwards to end in a filiform sterigma'. (English translation, pp. 242–3). Although the Tulasnes' 'genuine pycnidia' do not resemble the pycnidia we have obtained in culture, it is possible that the further stylospores which they describe are the same. Saccardo's *Hendersonia herpotricha* (*Sylloge Fungorum*, **2**, 352), with stylospores 8-septate, measuring 36 × 6 μ, does not match the conidia we have obtained.

McAlpine (1904) and Sprague (1935) have followed Saccardo in citing *H. herpotricha* as the conidial state of *Ophiobolus herpotrichus*. We have been unable to assign a name to the conidial state of *O. herpotrichus*. *Septoria arctica* Berkeley & Curtis (1860) originally described from *Dupontia fischeri* (see Sprague, 1950) has a filiform terminal appendage to the spore, but comparison with the type specimen in Herb. Kew. showed that it is not *O. herpotrichus*. Possibly the conidial state could be accommodated within the form-genus *Urohendersoniella* Petrak (1955), but it seems unnecessary to make a new combination.

Leptosphaeria luctuosa Niessl, in Saccardo, *Nuovo G. bot. ital.* **7**, 321, 1875

Ascospore isolations of this fungus have produced *Phaeoseptoria* conidia, and perithecia have also been found in cultures started from single pycnospores. The description is based on Herb. Sheffield No. 1765, on *Dactylis glomerata*.

Perithecia: associated with a reddish brown coloration of the tissues, single or in groups on nodes and leaf sheaths, black, globose, immersed, with only the neck penetrating to the surface, 150–200 μ diam., wall about 10–15 μ thick below, thicker above. *Asci*: numerous, bitunicate, club-shaped, 8-spored, 100–120 × 12–14 μ. *Ascospores*: biseriate in the upper portion of the ascus, uniseriate below, fusoid, curved or straight, 5-septate, slightly constricted at the septa, third cell enlarged; at first hyaline with a prominent guttule in each cell, later yellowish brown in colour, 28–36 × 5·5–6·5 μ.

Niessl's fungus was described from *Zea mays*. Unfortunately type material

Table 1. *Dimensions* (μ) *of some collections of* Ophiobolus herpotrichus *which have been grown in culture*

Herb. Sheffield no.	Host	Asci on host	Ascospores on host	Conidia on host	Conidia in culture
1758	*Agropyron repens*	150–197 × 10–11	136–164 × 2–3	—	68–84 × 3·5–4·5
1759	*Zerna ramosa*	182–204 × 10–11	144–184 × 2–3	—	72–104 × 3·5–4
1760	*Z. ramosa*	142–186 × 10–11	—	—	78–102 × 3–4
1761	*Agropyron repens*	—	—	—	78–102 × 3–4
1818	*A. repens*	112–168 × 8–10	120–168 × 2·5–3	69–102 × 3·5–4	—
1816	*A. repens*	—	—	71–86 × 3–4	71–84 × 3–4
1815	*A. repens*	—	—	73–116 × 3–4	—
1817	*A. repens*	147–205 × 9–11	137–170 × 2·5–3	—	71–119 × 3·5–4
1763	*Wheat*	144–195 × 9–11	132–166 × 2·5–3	—	74–112 × 3·5–4·5
1813	*Arrhenatherum elatius*	166–231 × 9–11	138–197 × 2·5–3	—	—
1812	*Bromus sterilis*	128–206 × 9–10	129–168 × 2·5–3	—	72–122 × 3·5–4·5
1814	*Holcus mollis*	162–224 × 9–11	126–198 × 2·5–3	—	82–121 × 3·5–5
1757	*Agropyron repens*	136–176 × 9–10	124–172 × 2·5–3	—	—
1799	*Festuca rubra*	140–170 × 9–10	110–140 × 2·5–3	—	—
1811	*Agropyron elatius*	106–180 × 8–10	126–140 × 2–3	—	—

Table 2. *Dimensions* (μ) *of ascospores and conidia of some collections of* Leptosphaeria luctuosa *on the host and in culture*

		Host			Culture		
Specimen no.	Host	Ascospores	Conidia	Transverse septa in conidia	Ascospores	Conidia	Transverse septa in conidia
1350b	*Holcus mollis*	—	62–74 × 4–5	7–12	26–32 × 4–5	—	—
1401	*Calamagrostis epigeios*	—	56–60 × 3·5–4	6–7	26–30 × 4·5–6	62– 80 × 2–2·5	7–9
1771	*Agropyron repens*	—	72–90 × 3–4	7–13	30–37 × 5–6	58– 88 × 3–3·5	8–10
1765	*Dactylis glomerata*	28–32 × 5·5–6	—	—	28–30 × 5–6	86–120 × 2–3·5	7–15
1768	*Agropyron repens*	30–34 × 5–6	—	—	—	60–104 × 3–4	8–15
1769	*A. repens*	28–36 × 5·5–6·5	—	—	26–30 × 5–6	40– 72 × 3–4	7–10
1772	*A. repens*	28–32 × 5–6	—	—	—	64– 80 × 2·5–3·5	7–8
1713	*Ammophila arenaria*	26–32 × 5–6	—	—	—	52– 88 × 2·5–4	7–12
1773	*Glyceria maxima*	26–30 × 5·5–6	—	—	28–32 × 5–6	74– 90 × 2·5–3	7–11

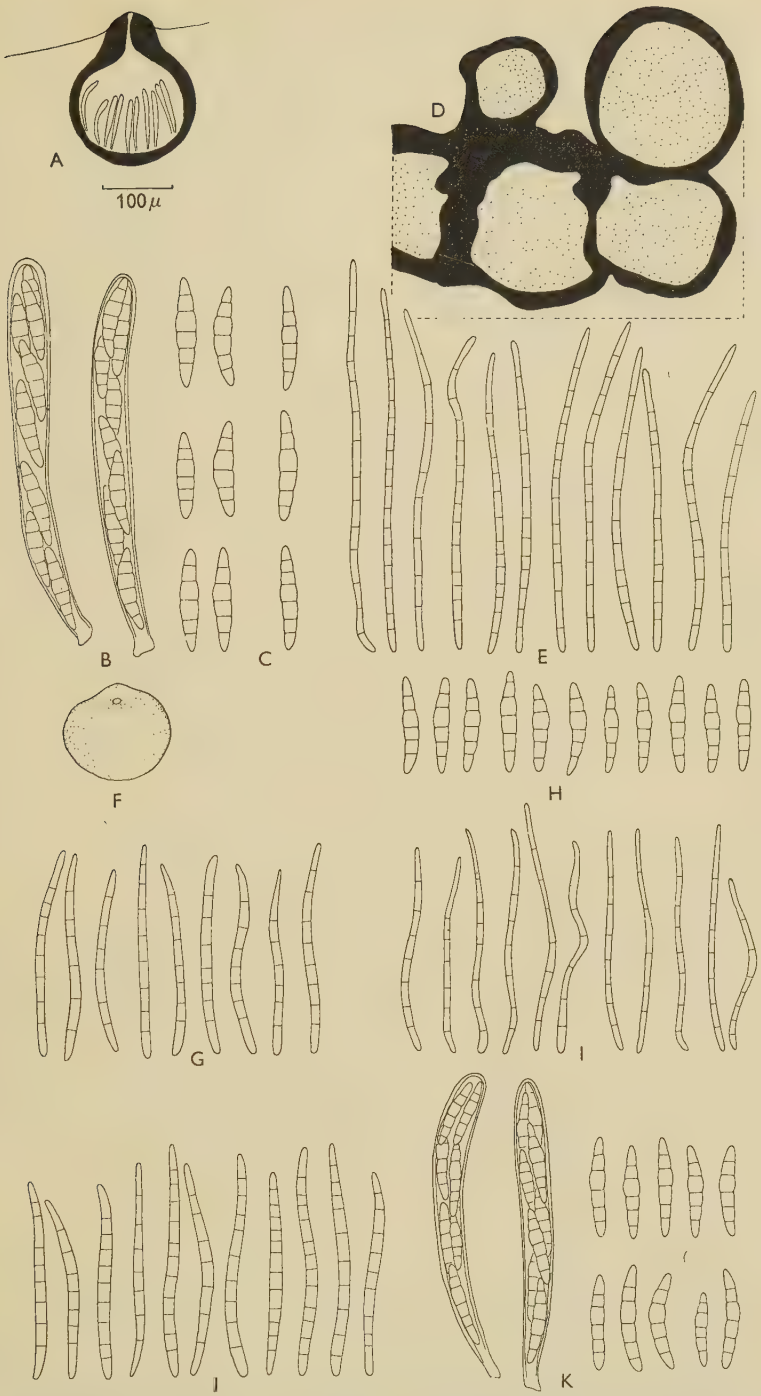

Fig. 2. *Leptosphaeria luctuosa*. A, L.S. *Dactylis* leaf sheath and perithecium (1765); B, asci and ascospores from host (1765); C, ascospores from culture (1765); D, section of oat agar culture derived from a single ascospore showing pycnidia (1765), E, pycnospores from culture (1765); F, pycnidium from *Calamagrostis epigeios* (1401); G, pycnospores from *C. epigeios*; H, I, ascospores and pycnospores from culture started from a pycnospore (1401); J, pycnospores from *Holcus lanatus* (1350b); K, asci and ascospores from culture started from a pycnospore (1350b).

is not available either from the Saccardo Herbarium at Padua or Niessl's Herbarium at Münich. Berlese (1894) figured and described a fungus claimed to be from the original specimen with asci 150–160 × 18–20 μ and dark brown ascospores 35–40 × 10–12 μ, but this is probably not the fungus which Niessl described.

Cultures. Single ascospore cultures were prepared on oat agar on 28 June 1955. The colonies grew fairly rapidly, forming a black-coloured mycelium at the surface of the medium, with pale grey or white aerial hyphae and orange to rose tints at the upper end of the slopes. Pycnidia and perithecia were found within 2 months, frequently in contact with the glass of the culture tube. The perithecia resembled those on the host, but the ascospores were slightly narrower, measuring 28–32 × 5–6 μ. The pycnidia were irregularly globose, variable in size, up to 350 μ diam., thick-walled (30–40 μ). Pycnospores were formed from an inner layer of hyaline cells: at maturity they oozed from the mouths of the pycnidia in conspicuous brown masses. Mature pycnospores were filiform, straight or curved, pale brown, 9–15 septate, not constricted at the septa, wider below, tapering gradually upwards, 86–112 × 2·5–3 μ.

Pycnidia on the host. Pycnidia have been collected on various hosts (see Table 2) which have been shown by culture experiments to be referable to *Leptosphaeria luctuosa.* Cultures started from single pycnospores have produced perithecia resembling those started from ascospores. The pycnidia are small black, globose, about 150 μ diam., single, between the veins of the grass leaf. The pycnospores are filiform, 6–13 septate, tapering above, 50–90 × 3–5 μ. Measurements of individual collections are given in Table 2. As shown in the table the behaviour of different isolates of the fungus is variable. Cultures started from single ascospores or single conidia may yield either conidia or perithecia or both.

Whilst it is tempting to try to assign a name to the conidial stage, this is rendered hazardous by the fact that *L. fuckelii*, *L. nigrans* and *L. microscopica* produce very similar conidia in culture (see Webster, 1955). On the whole, however, conidia of *L. luctuosa* are slightly narrower than those of *L. nigrans* and *L. microscopica.* Hughes (1949) has queried whether *Septoria alopecuri* var. *calamagrostidis* is the conidial stage of *L. nigrans.* In our opinion it is equally likely to be the conidial stage of *L. luctuosa.*

Perithecia of *L. luctuosa* have been collected throughout the year on basal internodes and on collapsed stems of various grasses, but most frequently collected during spring and early summer.

Leptosphaeria fuckelii. Niessl, in Voss (1882), *Ost. Bot. Z.* **32**, 357

Perithecia of *L. fuckelii* are very common in Britain on the leaf sheaths and stems of *Phalaris arundinacea*, and most of our collections have been made in spring and early summer on the basal internodes on collapsed stems, but perithecia have also been collected in autumn in the yellowing upper leaf sheaths. Although reported from various other grasses we have no collections on hosts other than *Phalaris.* Isolates of the fungus have produced conidia of the *Phaeoseptoria* type. The description below is based on Herb. Sheffield No. 1824A.

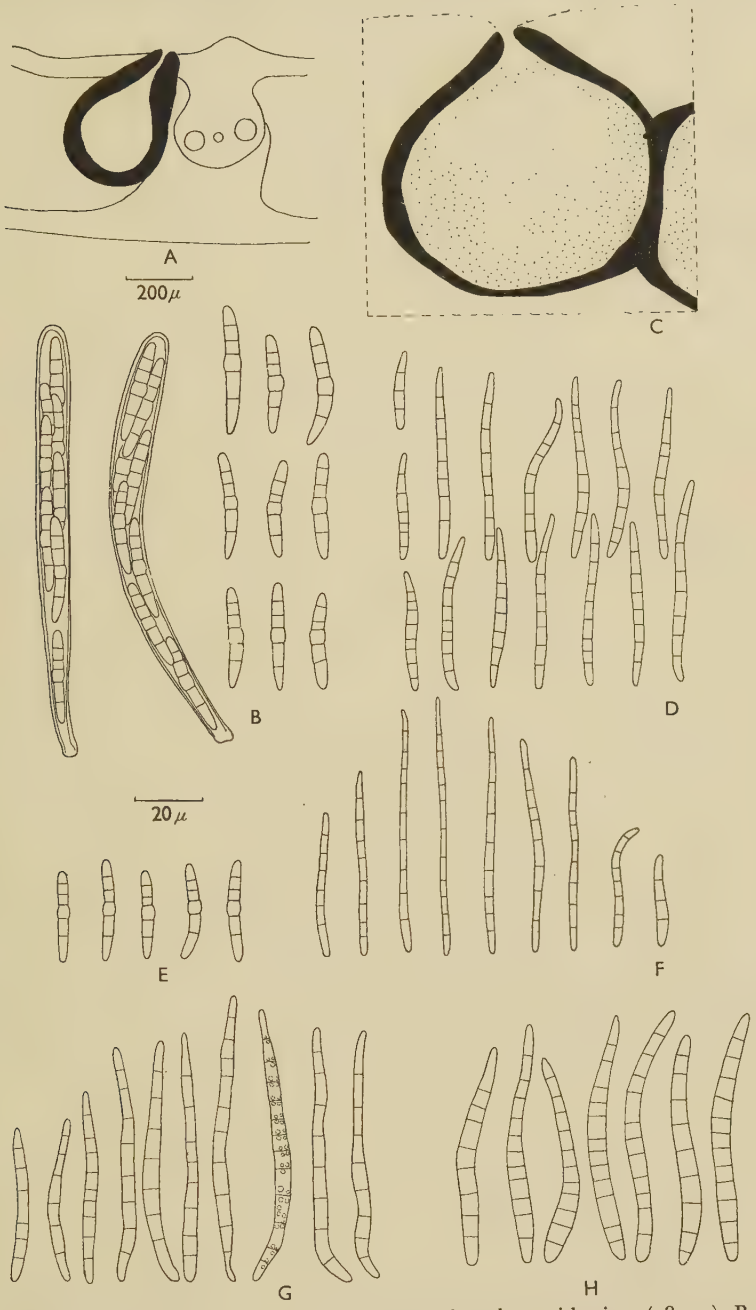

Fig. 3. *Leptosphaeria fuckelii.* A, T.S. *Phalaris* leaf sheath and a perithecium (1824a); B, asci and ascospores (1824a); C, section of oat agar culture and a pycnidium (1824a); D, pycnospores from culture (1824a); E, ascospores (1798); F, pycnospores from a single ascospore culture (1798); G, pycnospores from *Phalaris* (1895), cultures prepared from this pycnospore suspension yielded perithecia; H, *Septoria bromi* var. *phalaridis* Trail. Pycnospores from the type collection.

Perithecia: subepidermal, arranged in rows between the veins, black, globose, about 200 μ diam., penetrating the epidermis by a short truncate neck. Wall of perithecium 15–30 μ thick, composed of about five layers of brown, polygonal cells 8–12 μ wide on the outside, lined by smaller hyaline cells. *Asci*: numerous, club-shaped, bitunicate, 8-spored, 106–120 × 9–10 μ, separated by numerous filiform paraphyses. *Ascospores*: biseriate in the upper part of the ascus, uniseriate below; yellow, straight or curved, 5-septate, 26–34 × 4.5–6·5 μ; upper half of spore cylindrical, composed of three cells of equal width, 4th cell enlarged; lower half of spore tapering, composed of two cells longer than the rest (see Fig. 3B). Occasional 6-septate spores are also found.

The specimen has been matched with a collection authenticated by G. von Niessl in Herb. Kew. (Rabenhorst-Winter, Fungi Europaei No. 3259), on *Calamagrostis montana*, and with various other collections by Niessl, from the Botanische Staatsammlung, München.

Cultures were prepared from single ascospores and single asci and transferred to oat agar slopes. Pycnidia and perithecia have been found in culture from single ascospores, within 2 months. On oat agar the isolates produce grey to black hyphae at the surface of the medium with white to rose-coloured aerial mycelium at the upper end of the culture tube. Pycnidia are usually formed in contact with glass of the tube and at the surface of the medium, singly or in groups. They are globose, at first pink, later brown to black, variable in size, 200–1000 μ diam. The wall of the pycnidium is 20–40 μ thick and made up of dark polygonal cells on the outside and hyaline polygonal cells within. Pycnospores arise from cylindrical open-ended, phialide-like structures lining the pycnidium, and ooze out in brown masses from the pycnidia, but ostioles are inconspicuous. Pycnospores are pale brown in colour, cylindrical to narrowly obclavate, tapering slightly upwards to a rounded tip, truncate below, 3–9 septate, not constricted at the septa, 30–81 × 3–4 μ. Measurements of other ascospore isolates are given in Table 3. Conidia obtained in these cultures range in size from 30–91 × 2·5–4 μ, with 3–15 transverse septa.

Pycnidia on the host. In the early autumn and less frequently in the spring, pycnidia resembling those obtained in culture can be found associated with perithecia. Isolates have been prepared from pycnospores from many collections, and in several isolates perithecia of *L. fuckelii* have been found either alone or with pycnidia. The description is based on Herb. Sheffield 1895, isolates from which have produced *L. fuckelii* perithecia (Fig. 3G).

The pycnidia on the host are inconspicuous, distributed between the veins, pale brown, globose, 120–200 μ diam. The pycnospores (from the single pycnidium from which isolates were prepared) resemble those described above and are brown in colour, 7–12 septate, 64–90 × 5 μ, mostly 60–70 × 4–4·5 μ. Measurements from some other conidial collections which have yielded perithecia in culture are given in Table 3. The range of size of conidia from the host is 48–94 × 4–6, with 7–15 transverse septa. Although we have many other collections which probably represent *L. fuckelii* conidia, dimensions are not included in Table 3 because isolates from these collections have so far failed to produce *L. fuckelii* perithecia. Because *L. microscopica*, which has been collected on *Phalaris*, forms pycnidia

very similar to those of *L. fuckelii* (Webster, 1955), we cannot identify these collections with confidence in the absence of perithecia in culture. For this reason also the question of a name for the conidial state of *L. fuckelii* must remain open. It seemed possible that the fungus described by Trail (1887) as *Septoria bromi* var. *phalaridis* might represent the conidia of *L. fuckelii*. Trail's description runs: 'On dead leaves of *Phalaris arundinacea*, near Aberdeen, in September, sporidia 53–65 × 3½–4, septa 8–15'. Through the kindness of Prof. J. R. Matthews we have examined Trail's material,

Table 3. *Dimensions* (μ) *of some collections of* Leptosphaeria fuckelii *which have been grown in culture*

	Host			Culture		
Specimen no.	Ascospores	Conidia	Number of transverse septa in conidia	Ascospores	Conidia	Number of transverse septa in conidia
1798	28–31 × 4–5	—	—	—	30–78 × 3–3·5	3–15
1824a	26–34 × 4·5–6·5	—	—	—	30–81 × 3–4	3–9
1825	26–32 × 4–5	—	—	31–37 × 4–5	31–72 × 2·5–3·5	3–9
1826	27–30 × 4–4·5	—	—	—	60–74 × 3·5–4	8–13
1883b	—	48–70 × 3·5–5·5	7–8	24–27 × 4–5	—	—
1895	—	64–90 × 3–5	7–12	24–30 × 4–4·5	45–72 × 3–5	7–8
1898	—	50–88 × 4–6	7–11	26–32 × 4–4·5	46–82 × 3·5–5	7–15

including his slides, but we are of the opinion that this fungus is not *L. fuckelii*. The pycnidia are seated on brown lesions on green leaf blades, and contain hyaline, vermiform conidia measuring 48–80 × 5–6 μ, with 5–14 septa (see Fig. 3H). The conidia are thus considerably wider than in Trail's description. Sprague (1943), apparently without having examined Trail's material, proposed the new combination *Phaeoseptoria phalaridis* (Trail) Sprague. Sprague's description, based on a collection by W. B. Cooke (Oregon State College No. 17) refers to 'sphaerical pycnidia, composed of light golden-brown, large celled mycelia from which cuspidate pycnospores arise from creeping hyphae. The spores are 8–13 septate, filiform, 70–90 × 2·5–4·5 μ, somewhat constricted at the septa.' It is possible that this collection represents *L. fuckelii* conidia. Unfortunately this specimen can no longer be found in the Herbarium of Oregon State College (W. B. Cooke, *in litt.*).

Leptosphaeria pontiformis (Fuckel) Sacc., *Sylloge Fungorum*, **2**, p. 78

Perithecia of *L. pontiformis* have been found on basal internodes of *Agropyron repens* and *A. caninum* from January to April. Ascospore isolates have yielded conidia of the *Hendersonia* type, and also on one occasion of the *Phoma* type. The description below is based on a collection on *A. caninum*, Herb. Sheffield No. 1797.

Perithecia: arising from a dark brown mycelium about 4 μ wide which surrounds the base of perithecia, single or more usually in rows, 200–250 μ diam., black, globose, superficial, raising a thin strip of epidermis and piercing it by short truncate necks. The wall of the perithecium is 20–30 μ

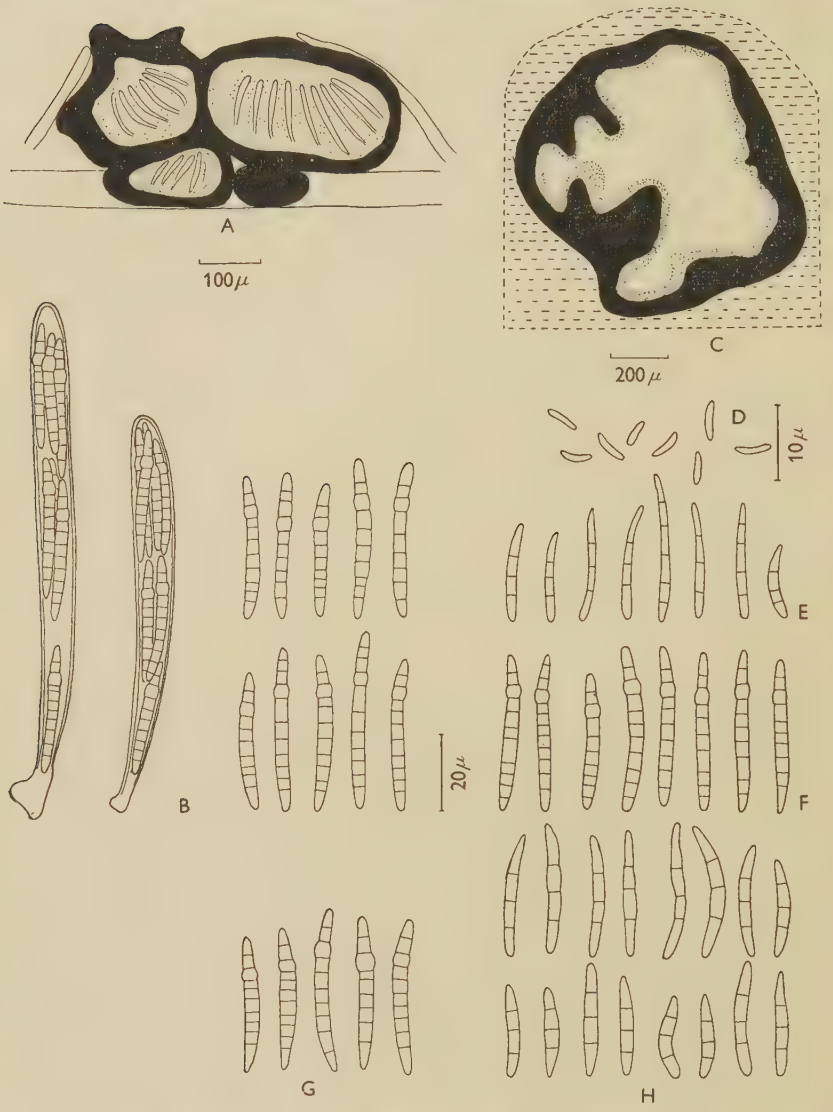

Fig. 4. *Leptosphaeria pontiformis*. A, L.S. *Agropyron* stem and perithecia (1797); B, asci and ascospores (1797); C, section of oat agar culture with *Phoma* type pycnidia (1797); D, *Phoma* type pycnospores (1797); E, *Hendersonia* type pycnospores from culture (1797); F, Ascospores from Fuckel's Fungi Rhenani 782; G, ascospores (1907); H, *Hendersonia* type pycnospores (1907).

thick, and made up of dark brown, rounded or polygonal cells up to 8 μ wide. *Asci*: numerous, cylindrical or club-shaped, bitunicate, 8-spored, 90–100 × 10–12 μ, separated by filiform paraphyses. *Ascospores*: quadriseriate in the upper part of the ascus, often less regular below, cylindrical, straight or slightly curved, pale yellow, 8–12 septate (typically 10-septate), occasionally somewhat constricted at the septa, the 3rd or 4th cell enlarged, end cells rounded; 34–44 × 4–5 μ (width of widest cell).

The fungus has been matched with Fuckel's Fungi Rhenani No. 782 from Herb. Kew. (Fig. 4F). Winter's (1887) description based on another portion of this exsiccatum mentions 16-septate spores, but we have only seen up to 12 septa. Measurements from other collections are given in Table 4.

Table 4. *Dimensions* (μ) *of some collections of* Leptosphaeria pontiformis *which have been grown in culture*

Specimen no.	Host	Asci on host	Ascospores on host	Conidia in culture
259	*Agropyron repens*	110–134 × 12–16	38–48 × 4–4·5	—
1658	*A. repens*	110–130 × 10–12	40–50 × 4–5	Failed to fruit
1793	*A. repens*	100–120 × 10–11	32–40 × 4–5	Failed to fruit
1797	*A. caninum*	90–100 × 10–12	34–44 × 4–5	20–32 × 2–3
1907	*A. repens*	110–130 × 10–12	38–44 × 4–5	20–30 × 3–4
1909	*A. repens*	110–130 × 10–12	38–42 × 4–4·5	18 × 32 × 3–3·5

Cultures. Isolates from single ascospores were grown on oat agar slopes. The cultures were uniformly dark brown to black in colour with grey-green aerial mycelium. Pycnidia were found within 3 months in certain isolates. Two types were found in isolates from Specimen 1797.

(*a*) *Hendersonia* type. The pycnidia were black globose, 200–300 μ diam. and produced narrowly obclavate, pale brown, smooth unconstricted, straight or slightly curved pycnospores with 3–5 (typically 3) transverse septa; 20–32 × 2–3 μ. Measurements from other isolates are given in Table 4.

(*b*) *Phoma* type. These pycnidia were larger (up to 800 μ diam.), black, globose, often multi-loculate structures. The wall was up to 40 μ thick and made up of dark rounded cells or interwoven hyphae about 5 μ wide. The inner wall of the pycnidium is lined by branched sporophores, terminating in tapering phialides which abstrict numerous smooth, hyaline allantoid or rod-shaped spores, 3·5–6 × 1 μ.

Fuckel (1870) claimed that *Torula rhizophila* Corda was the conidial state of *Leptosphaeria pontiformis*. His statement was not based on cultures, and Corda's fungus bears no resemblance to our isolates.

Leptosphaeria eustomoides Sacc., *Nuovo G. bot. ital.* **7**, 319, 1875

Cultures derived from ascospores have yielded pycnidia of the *Hendersonia* type. Similar conidia have been collected on grasses and their identity confirmed by obtaining perithecia in cultures derived from conidia. The description below is based on Herb. Sheffield No. 1755 on *Dactylis glomerata*.

Perithecia: single or in groups between the veins of the leaf sheath, black, globose with flattened truncate neck, 130–190 μ diam., wall only 10–15 μ thick at the base of the perithecium, composed of dark, polygonal, thick-walled cells up to 30 μ wide. *Asci*: numerous, cylindrical to club-shaped, bitunicate, 8-spored, 60–70 × 6–8 μ. *Ascospores*: biseriate, fusoid, yellowish brown, 3-septate, slightly constricted, the second cell enlarged, end cells pointed; 18–20 × 3–4·5 μ. Measurements from other collections are given in Table 5.

The specimens have been compared with a collection from Saccardo's Herbarium No. 100 'Aux gaines et aux feuilles d'une Phragmites', Mentone 10 May 1875. The asci and ascospores are somewhat more robust

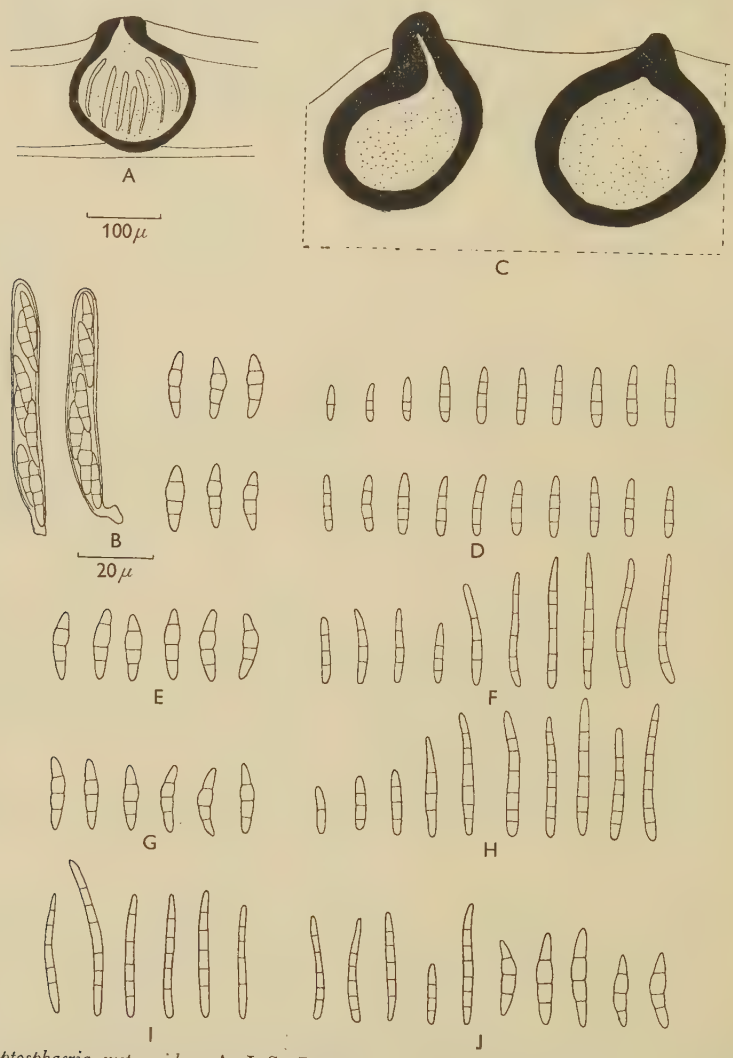

Fig. 5. *Leptosphaeria eustomoides*. A, L.S. *Dactylis* leaf sheath and perithecium (1755); B, asci and ascospores (1755); C, section of oat agar culture and pycnidia (1755); D, pycnospores from culture derived from a single ascospore (1755); E, ascospores from *Agropyron* (1774); F, conidia produced in culture derived from a single ascospore (1774); G, ascospores from *Agropyron* (1777); H, conidia produced in culture derived from a single ascospore (1777); I, conidia from *Agropyron* (1778); J, conidia and ascospores produced in culture derived from a single conidium (1778).

than in our collections, measuring 64–86 × 10–12 and 18–21 × 4·5–5·5 μ, respectively (see Fig. 6). However, Saccardo's (1875) description of the type specimen on *Andropogon ischoemi* gives ascospore measurements

20 × 4·5 μ. Unfortunately the type specimen is no longer available. Berlese's figure (Berlese, 1894, Tab. XLII, Fig. 4) from the type specimen matches well with our material. Berlese's measurements are: asci 55–65 × 8–10, ascospores 18–20 × 4–5 μ.

Cultures. Cultures derived from single ascospores or single asci were grown on oat agar slopes. The colonies were grey to black in colour, with brown coloration in the medium and sparse white aerial hyphae. Pycnidia were found within 6 weeks at the surface of the medium. They were single or compound structures, black, globose, with very short necks and with walls 20–25 μ thick, the outer layers composed of dark brown polygonal cells 10–15 μ wide. The pycnospores arise from cylindrical or tapering sporophores borne on hyaline thin-walled cells lining the pycnidia. Mature pycnospores are pale brown in colour, obclavate, truncate at the base, tapering slightly to the rounded apex, 3-septate, 13–18 × 2·5–3 μ. Measurements of pycnidia from cultures derived from other collections are listed in Table 5. Perithecia and pycnidia were found in some isolates. In some of these cultures, the pycnospores are up to 7-septate, with a size range of 18–40 × 2–3·5 μ.

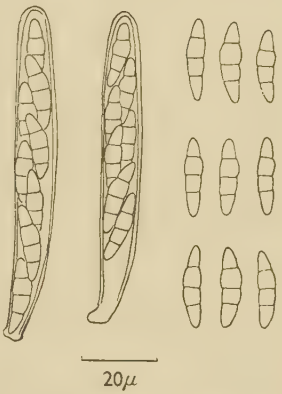

Fig. 6. *Leptosphaeria eustomoides.* Asci and ascospores from Herb. Saccardo No. 100.

Pycnidia on the host. Similar pycnidia have been collected on *Agropyron repens* (Herb. Sheffield No. 1778). The pycnospores were 30–44 × 2–3 μ with 3–6 transverse septa. Cultures derived from pycnospores yielded both perithecia and pycnidia.

Table 5. *Dimensions* (μ) *of some collections of* Leptosphaeria eustomoides *which have been grown in culture*

Specimen no.	Host		Culture	
	Ascospores	Conidia	Ascospores	Conidia
1755	18–20 × 3–4·5	—	—	13–18 × 2·5–3
1774	18–23 × 3·5–4·5	—	20–24 × 3·5–4	18–10 × 2·5–3
1775	19–23 × 4–4·5	—	—	20–29 × 2·5–3·5
1776	17–22 × 3·5–4	—	—	18–29 × 2·5–3·5
1777	18–21 × 3·5–4	—	20–26 × 4–4·5	19–31 × 2·54
1778	—	30–44 × 2–3	20–26 × 4–4·5	22–37 × 2–3

Discussion

The close similarity between the conidia of various graminicolous Leptosphaerias (e.g. *L. microscopica*, *L. nigrans*, *L. luctuosa* and *L. fuckelii*) serves to emphasize the difficulties we have pointed out earlier (Webster, 1955) in finding names for these conidial states. Without resort to cultures we find it extremely difficult to separate these conidial forms, and thus even where type material of a pycnidial state is available it may be difficult to assign a name with confidence. It is interesting to speculate on the

reasons for the similarity of the conidia in these different fungi and it would be interesting to know if the *Phaeoseptoria* type of conidium, which is common on grasses and sedges, is especially advantageous for dispersal and attachment to these hosts.

It is a pleasure to thank all who have enabled us to study specimens in their keeping: Prof. C. Cappelletti, Dr R. W. G. Dennis, Prof. J. T. Matthews and Dr J. Poelt. We are also grateful to Dr W. B. Cooke and Dr R. Sprague for information about material of *Phaeoseptoria phalaridis*, and to Dr E. Müller for information about Niessl's herbarium. We are indebted to the University of Sheffield Research Fund for a grant in aid of this work.

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A FURTHER CONTRIBUTION TO THE PHYCOMYCETE FLORA OF GREAT BRITAIN*

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(With 2 Text-figures)

This continuation of an earlier study of the Phycomycetes of Great Britain has been confined largely to soil inhabitants. Collections were made from over ninety different sites in England, Wales and Scotland. Forty-three different species were identified and partial information on a number of others was obtained. Three are considered new species.

Sparrow (1936) recorded the results of a year's study of Phycomycetes of Great Britain, fungi for the most part collected in and around Cambridge from aquatic habitats. The present paper embraces the results of a study of the same group conducted during February to May 1956. Two different approaches from those employed in the earlier study were used: emphasis was placed upon soil inhabitants, and a much broader geographic area was covered. Indeed, so preponderantly were soil samples used as the source of the organisms, that his paper might well be sub-titled 'Some British Soil Phycomycetes'.

The methods used in recovery from soils were those commonly employed by modern investigators of these fungi and might be said to be noteworthy for both their simplicity and crudity. Soil samples were collected in small, new boxes, and the collecting tool was sterilized with alcohol after each sampling. The boxes were cellophane-taped shut and labelled. In the laboratory each sample was processed as follows. A generous tablespoon of soil was put into a deep sterile Petri dish of sterile water. There was sufficient of the latter to allow for a free water surface above the soil. Reversing the process, i.e. putting the water on to the soil, usually produced undue turbulence and an undesirable deposition of silt on the 'bait'.

In an effort to obtain fungi of differing nutritional requirements, each gross soil culture was 'baited' with a variety of materials. Uniformly throughout the present study the following were employed: cellophane; boiled grass; pine pollen; a split boiled hempseed; purified shrimp chitin; insect wings; defatted human hair (blonde); and snake-skin cast. These were sufficiently diverse to yield sometimes in a single culture an interesting variety of types. In the course of the study it became increasingly apparent that all the Phycomycetes present in a soil sample did not appear at the same time. Thus, there was observed to be a first 'wave' of invasion of the substrata by certain fungi, followed by a gradual replacement of these over a period of several weeks by entirely new, probably slower-growing forms. Hence, if the full potentialities of a culture are to be

* Contribution No 1068 Botany Department, University of Michigan.

realized, it should be carried along for at least a month, and probably longer. It is now realized that in the present investigation many forms were not recovered because of limitations of time.

It is gratifying to note that a comprehensive study of soil Phycomycetes has been initiated (Willoughby, 1956) in England which should be most productive.

FUNGI COLLECTED

Chytridiales

Olpidium gregarium (Nowak.) Schroeter. Abundant in rotifer eggs, in small lawn pool, 'Byron's Lodge', Grantchester, Cambs, 10 April 1956.

O. pendulum Zopf. In dead pine pollen grains. Soil, St Mary's Abbey, York, Yorks, 18 April 1956.

Rhizophydium sphaerotheca Zopf. Common on dead pine pollen grains. Soil, garden, 17 Barton Road, 27 February; St Botolph's churchyard, Cambridge, 7 March; poolside, Black Ditch, Cavenham Heath, Suffolk, 8 March; Cathedral Close, Canterbury, Kent, 27 March; field near Orsett, Essex, 30 March; Housesteads, Hadrian's Wall, Northumberland, 19 April; pasture, Ambleside, Westmorland, 21 April; moat, Caerlaverock Castle, Dumfries, Scotland, 20 April; sphagnum bog, Randy Pike, Hawkeshead, Lancs, 21 April (coll. J. W. G. Lund).

R. gibbosum (Zopf) Fischer. On protozoan cysts. Black Ditch, Cavenham Heath, Suffolk, 8 March.

R. pollinis-pini (Braun) Zopf. Dead pollen of pine. Soil, 17 Barton Road, Cambridge, 27 February.

R. hyperparasiticum Karling. Parasitic on hyphae and oogonia of a sterile Phycomycete. Soil, grounds of Battle Abbey, Hastings, Sussex, 29 March.

R. keratinophilum Karling. Abundant on hair. Soil, 17 Barton Road, Cambridge, 27 February; rear of Botany School, Cambridge, 29 February; St Botolph's churchyard, Cambridge, 27 March (also on snake-skin); grounds of Battle Abbey, Hastings, Sussex, 29 March; field south of Ely and Bishop's garden, Ely, Isle of Ely, 10 April; near Crooklands, Westmorland, 21 April; Housesteads, Hadrian's Wall, Northumberland, 19 April; near Dumfries, Scotland, 20 April; pasture, Ambleside, Westmorland, 21 April.

R. granulosporum Scherffel. Parasitic on *Tribonema* sp. Black Ditch, Cavenham Heath, Suffolk, 8 March.

R. elyensis sp.nov. On snake-skin bait. Soil, Cathedral Close, Ely, Isle of Ely, 10 April.

This species occurred in fair abundance on snake-skin used as bait. At maturity, the sporangium is distinctly angular (Fig. 2I, L, M). This shape is no doubt due to the presence of five or more discharge papillae Fig. 2J, K). The latter are, however, scarcely visible on the thin wall of the sporangium. The rhizoids are endobiotic, much branched and arise from a single more or less elongated axis. The sporangium is sessile on the substratum and young developmental stages all indicate for it a *Rhizophydium*-type of development.

Rhizophydium elyensis sp.nov. (Fig. 2 I–M)

Sporangium sessile, somewhat spherical at first but soon becoming distinctly and irregularly polygonal, 23–25 μ in largest diam., wall thin, smooth, colourless and rigid; rhizoids delicate, moderately well developed, branched, arising from base of sporangium or from a main axis; zoospores with an eccentric globule and posterior flagellum, passively emerging through 2–5 pores formed upon the deliquescence of scarcely visible papillae, initiating individual movement out in the water; resting spore not observed.

Saprophytic on snake-skin used as bait. Soil, Ely, Isle of Ely, 10 April 1956.

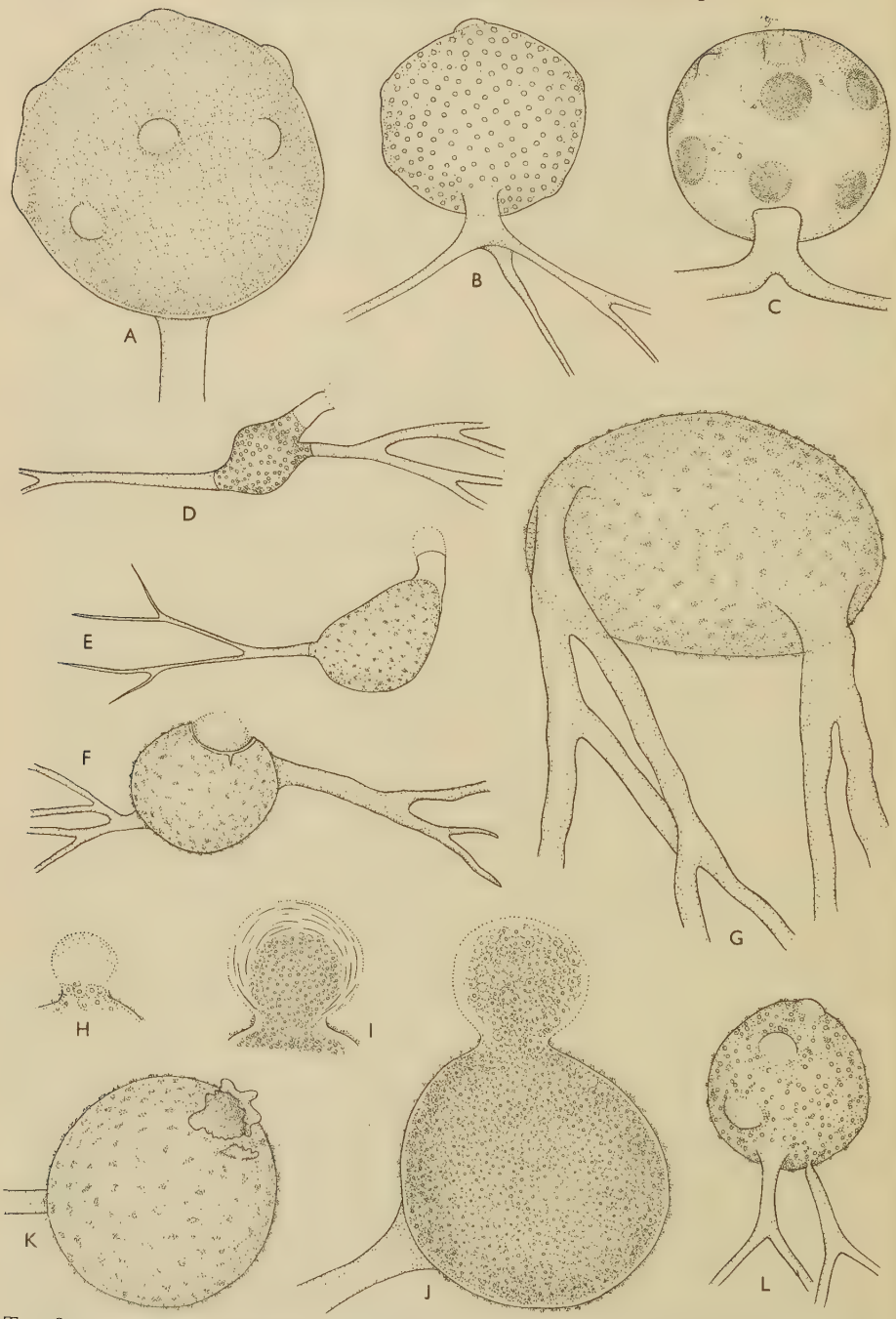
Sporangium sessile, primum subsphaericum, cite distincte et irregulariter polygonum, 23–25 μ diam., membrana tenui, laevi, pellucida, rigida; rhizoideis tenuibus, ramosis, modice extensis, e basi sporangii vel ex axe primario brevi orientibus. Zoosporae globulo excentrico et flagello postico praeditae, per 2–3 poras delisquescentia papillae vix visibilis formatas languide emergentes, in aqua mox motantes. Sporae perdurantes nondum observatae.

Saprophyticum in exuvio serpentis in aqua supra solum natante, ex loco 'Ely, Isle of Ely', Britannia.

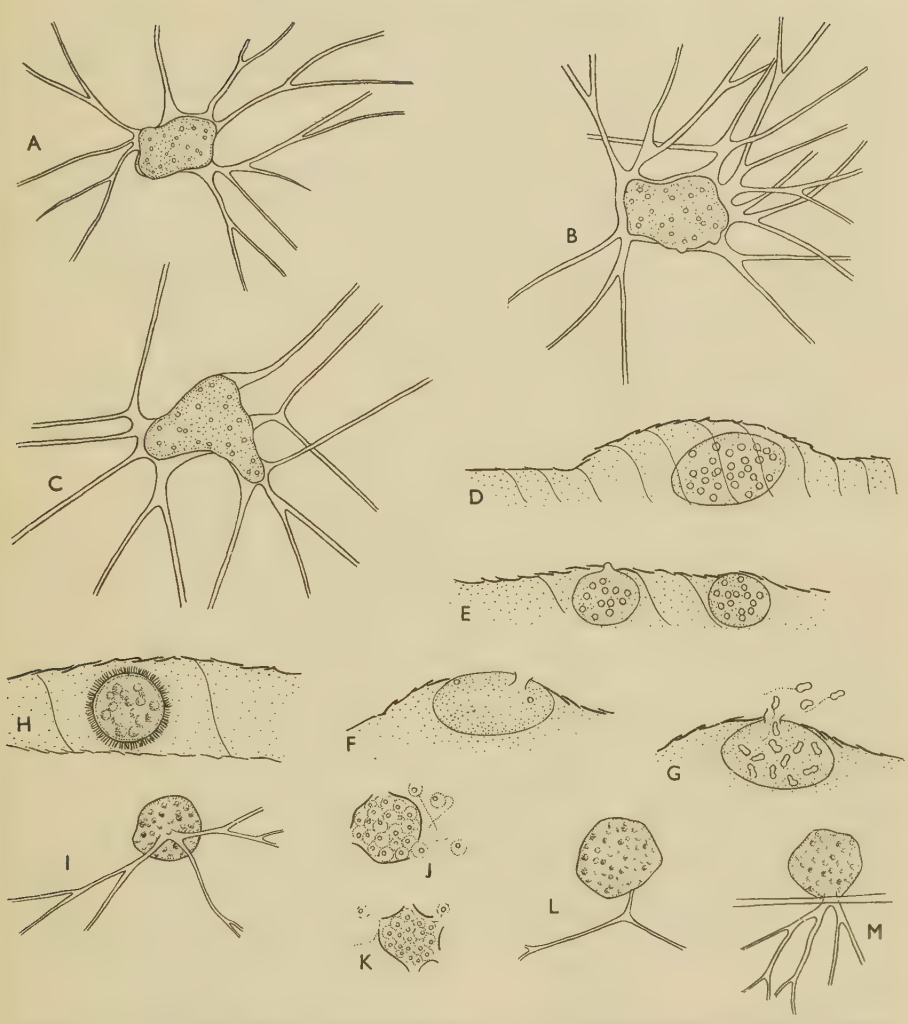
R. stipitatum sp.nov. On snake-skin bait. Soil, Black Ditch, Cavenham Heath, Suffolk, 8 March; gorse field, Annan, Dumfries, Scotland, 20 April.

This interesting form was recovered in five different collections from Cavenham Heath, where it apparently abounds. It has several very distinctive features, chief of which is the usual formation of a stout extramatrical stalk up to 40 μ long by 16 μ diam., at the tip of which the multiporous spherical, or somewhat angular sporangium is borne (Fig. 1 A, B). The latter structure is unusually large, ranging from 55 to 107 μ diam. and bears at maturity up to eight sometimes elevated broad (12–20 × 5 μ high) discharge papillae. The posteriorly uniflagellate zoospores are exceedingly numerous, 5 μ diam. and bear a small basal, colourless globule. They escape rapidly through the broad pores formed at the deliquescence of the papillae (Fig. 1 C). No resting spores have been found.

The present organism by its stipitate character invites comparison with the recently described *Phlyctochytrium stipitatum* Ookubo (1954). In this fungus, the stalk is regarded as a columnar 'exobiotic' apophysis, hence its assignation to *Phlyctochytrium*. In our fungus, the stalk, which is not always present, is not so regarded. Whether assigning a fungus with such a structure to *Rhizophydium* does too much violence to the concept of that genus is, of course, open to question. Ookubo raises the same question with respect to *Phlyctochytrium*. Other stalked sporangial types have been assigned to *Rhizophydium* in the past. Regardless of generic considerations, our fungus is quite distinct from the Japanese form. The body of the sporangium is considerably larger, 55–107 μ diam. compared with 45–55 μ, there are up to eight broad discharge pores distributed over the surface, not a single subapical one, the zoospores are distinctly smaller, 5 μ diam., compared with 20 × 23 μ, and the basal stalk is thin-, rather than thick-walled.



Text-fig. 1. A–C. *Rhizophydium stipitatum* sp.nov. A, B, nearlymature stalked sporangia with discharge papillae. C, nearly empty sporangium, eight discharge pores visible, one distinctly elevated; a few zoospores remain. D, E. *Rhizophlyctis* sp. F–K. *R. ingoldii* sp.nov. on chitin. F, developing sporangium with crustose wall and broad apical papilla. G, very large cushion-like sporangium. H–J, stages in zoospore discharge. K, empty sporangium with fractured wall through which discharge papilla emerged. L, *Rhizophlyctis* sp. with roughened wall and several discharge papillae. All × 380.



Text-fig. 2. A–C. *Rhizophlyctis* sp. from snake-skin (× 350), immature thalli. D–H. Keratinophilic endophytic fungus on human hair (× 350). D, E, mature sporangia lying in chambers in swollen hair. F, discharged sporangium. G, discharging sporangium. H, spiny resting structure. I–M. *Rhizophydium elyensis* sp.nov. on snake-skin (× 380). I, L, M, mature sporangia. J, K, discharging sporangia.

Rhizophydium stipitatum sp.nov. (Fig. 1 A–C)

Sporangium generally terminating a stalk up to 40 μ in length by 16 μ diam., occasionally sessile, spherical, 55–107 μ diam., becoming somewhat angular upon the formation of 6–8 or more 12–20 μ broad by 5 μ high papillae which are scattered over the surface, wall smooth, colourless; rhizoids arising from base of stalk (or of sporangium in sessile forms), running along surface of substratum as well as imbedded in it, coarse, up to 16 μ diam., much branched; zoospores spherical, 5 μ diam., with a

small basal colourless globule and long posterior flagellum, very numerous, emerging through broad sessile or somewhat elevated pores formed upon the deliquescence of the discharge papillae; resting spores not observed.

Saprophytic on snake-skin, Cavenham Heath, Suffolk, 8 March; Annan, Dumfries, Scotland, 20 April.

Sporangium immodice magnum, sphaericum vel subangulosum, 55–107 μ diam., in stipite crasso extramatricali interdum 40 μ longo, 16 μ crasso positum, multiporosum, maturitate non magis quam 8 papillas magnas emittantis ferens. Zoosporae numerosae postice uniflagellatae, 5 μ diam., globulum pellucidum includentes, per poras delisquescentia papillae formatas liberatae. Sporae perdurantes nondum observatae. Ex solo cultum in exuvio serpentis natanti in aqua; ex loco 'Cavenham Heath' dicto, Suffolk, et prope 'Annan, Dumfries', Scotia.

Differi a *Phlyctochytrio stipitato* sporangio grandiori, poris pluribus dispersis, non solitaria subapicali; zoosporis parvioribus; stipite tenuiter vallata, haud crasse.

Asterophlyctis sarcoptoides H. E. Petersen. On chitin bait. Sphagnum bog, Randy Pike, Hawkeshead, Lancs (coll. J. W. G. Lund) 21 April.

Some thalli had an undoubted *Entophlyctis*-like type of development; the majority, however, developed in typical *Rhizidium*-like fashion.

Phlyctorhiza peltata Sparrow. Snake-skin. Soil, 17 Barton Road, Cambridge, 27 February. See remarks under following species.

P. variabilis Karling. On snake-skin bait. Soil, near Crooklands, Westmorland, 21 April; Abergavenny, Wales, 11 April.

Although the material on snake-skin resembled the fungi shown by Karling (1947, figs. 1–20) it did not resemble either the other fungi figured by him or the strongly polycentric form he later described (Karling, 1951). The first-mentioned fungi would seem to be better accommodated in *Entophlyctis* rather than *Phlyctorhiza*, reserving the latter genus for types which, as emphasized by Miss Hanson (1946) in her comparison with *Rhizophlyctis*, but, strangely, not in the generic description, develop their sporangia by vesication of elements of the rhizoidal system. The polycentric form figured by Karling (1951, fig. 1) and termed *Phlyctorhiza variabilis*, is certainly a species of *Catenaria*, as its thallus and zoospores very clearly indicate. That *Phlyctorhiza peltata* Sparrow is a species of Miss Hanson's genus is also open to question, since it strongly resembles Karling's *P. variabilis*.

A possible member of this genus was found on an insect wing in a soil culture from Battle Abbey, Hastings, Sussex (29 March). Unfortunately, no zoospore discharge was observed. The thalli were somewhat triangular in shape, with two rhizoidal axes at the broad base and the cyst of the zoospore persistent at the narrower end. Discharge evidently occurred through a basal pore. No early developmental stages were found, but vesication of the main axes of a rhizoidal system might well have resulted in sporangium formation in this fungus.

Rhizophlyctis petersenii Sparrow. Grass bait. Soil, Abergavenny, Wales, 11 March; near Cowan Bridge, Lancs, 22 April.

R. rosea de Bary & Woronin. As anticipated, this proved the most ubiquitous chytrid present in soils, being collected from thirty-seven different samples. It was found exclusively on cellophane or grass used as bait. Although considerable variation was found in sporangial shape,

number of discharge papillae and rhizoidal axes, most of the collections fall well within the original concept of the species.

One form appeared in some abundance on grass in soil from near Orsett, Essex, which differed from the usual type in the constant possession of only a single broad tube terminating a somewhat pyriform sporangium (Fig. 1 D, E). Whether this should be regarded as a separate taxonomic entity from *R. rosea* is arguable, in view of the unsettled status of the numerous taxa already referred to the genus and the various forms described as *R.* (*Karlingia*) *rosea*.

R. ingoldii sp.nov. On chitin bait. Soil, 17 Barton Road, Cambridge, 27 February; Cathedral Close, Bishop's Garden, roadside field, Ely, Isle of Ely, 10 April; Abbots Barton Hotel gardens, Cathedral Close, Canterbury, Kent, 28 March; Dover Castle garden, Dover, Kent, 27 March; Battle Abbey, Hastings, Sussex, 29 March; woods, north of Harlow, Essex, 30 March; High Ongar, Essex, 30 March; Ledbury, Shropshire, 8 March; Selby Abbey grounds, Selby, Yorks, 17 April; moat of Caerlaverock Castle and field near Dornock, Dumfries, Scotland, 20 April.

As indicated, this remarkable and conspicuous chytrid was isolated from widely separated parts of Great Britain. It is a rapid and voracious destroyer of the chitin and usually occurs in such numbers on the substrate as to turn the latter a dark amber colour.

Its development is typically rhizidiaceous and the first-formed sporangia may be huge (up to 120 μ diam.) (Fig. 1 G, K, J) as compared with those growing later under more crowded conditions (Fig. 1 F). Further, the outer wall surface of the mature sporangium, especially in large examples, always possesses a characteristic amber-coloured granular incrustation. Small specimens may lack this almost completely. Another feature of this species is the presence of a large, broad, more or less apical discharge papilla. Occasionally, a second one may be formed, but this never approximates the other in size. Evidently, the incrusted wall of the sporangium is somewhat brittle, for as the discharge papilla develops, it pushes through and breaks the wall, leaving in some instances detached fragments (Fig. 1 K).

Characteristically, more than one usually very broad and coarse rhizoidal axis is found, although under crowded conditions a single broad, basal one is produced.

Zoospore discharge is initiated by the bulging out of the material of the conspicuous papilla and a simultaneous movement of the spore mass into it (Fig. 1 H-J). As the mass of passive spores moves through the broad exit pore, the papilla material continues to expand, becoming thinner and thinner as the spore mass enlarges. The latter in the course of 4–5 min. eventually approximates the sporangium in size and the many thousands of zoospores lie motionless, embedded in a matrix of slime derived from the material of the papilla. Movement of individual zoospores is then initiated on the periphery of the mass. Soon the entire group is in motion and very quickly the spores swim away with an irregular jerky movement. After a period of swarming they come to rest, resorb their flagella and germinate to form a single tube which branches at once or only after a period of elongation. One to several primary rhizoidal axes which may be up to 20 μ diam. originate in this manner.

The reproductive rudiment is formed from the enlarged body of the encysted zoospore. At maturity this may be spherical, subspherical or fusiform, sometimes strongly flattened and cushion-like (Fig. 1 G). Most often it is pyriform because of the broad, conspicuous papilla (Fig. 1 F). The wall, which in the young stages is hyaline or faintly amber and smooth, at maturity in well-developed sporangia is as stated previously, amber-coloured, crustose or granulated and rigid. The aforementioned discharge papilla is as much as 20 μ broad by 15 μ high and is composed of clear, refractive material. The cushion-shaped sporangia may be up to 120 μ diam. by 90 μ in height, with rhizoids as much as 17 μ in width. The more common spherical, subspherical or pyriform ones range from 50 to 90 μ diam. with rhizoids up to 12 μ diam., whereas small examples are 20 × 17 μ. No resting spores have been found as yet. That they exist is attested to by the ease of recovery of the fungus from time to time from dry, stored soil samples.

The species is named for Prof. C. T. Ingold of Birkbeck College, University of London, who has contributed so materially to the field of aquatic fungi.

Rhizophlyctis ingoldii sp.nov. (Fig. 1 F–K)

Sporangium spherical, subspherical, somewhat pyriform, and 50–90 μ diam., or occasionally cushion-shaped and up to 120 μ broad by 90 μ high, small examples 20 × 17 μ, the wall amber-coloured, rigid, and strongly incrusted with granulations on the outer surface, contents colourless, with a single broad conspicuous apical discharge papilla up to 20 μ diam. by 15 μ high in large sporangia, rarely two; with one to five main rhizoidal axes up to 20 μ diam. at the base of the sporangium, the rhizoids extensive and much branched; zoospores extremely numerous, 4–5 μ diam. with a minute eccentric globule, nuclear cap and posterior flagellum, the majority emerging in a mass embedded in the material of the discharge papilla, initiating flagellar activity after a few minutes and swimming away with an irregular, jerky movement, those remaining in the sporangium assuming motility there and escaping, resting spores not observed.

On chitin. Soil, Great Britain.

Sporangium subsphaericum vel subpyriforme, 50–90 μ diam., interdum depressum, non magis quam 120 μ latum, 80 μ altum (exempla parviora 20 μ lata, 16 μ alta), membrana straminea vel luteola, rigida, extus valde granulata, intus pellucidus; papilla singula (raro duabus) apicali interdum 20 μ lata, 15 μ alta, axibus primariis rhizoideis 1–5 haud magis quam 20 μ diam. ad basem sporangii, rhizoideis minoribus multum et extense ramosis; zoosporis numerosissimis, 4–5 μ diam., globulo minuto excentrico, cupula nucleari et flagello postico praeditis, pluribus emergentibus in massa cohaerentibus cum substantia gelatinosa papillae, sed mox motantibus, erratice natantibus. Sporae perdurantes nondum visae. In solo: culta in substrato chitinis. In Britannia.

Rhizophlyctis spp. The collections yielded a number of forms which were only incompletely observed, and hence their relationships could only be surmised. Two very characteristic types occurred repeatedly which seemed to be allied to *Rhizophlyctis*. Failure to induce zoospore discharge, however, made positive identification impossible.

The first of these (Fig. 2 A–C) was found on snake-skin bait in soil from

the following localities: 17 Barton Road, Cambridge, 27 February; Castle grounds, Rochester, Kent, 26 March; Cathedral Close, Canterbury, Kent, 27 March; banks of River Stour by Richborough Castle, Kent, 28 March; esplanade, Deal, Kent, 28 March; Chepstow, Monmouthshire, 9 April; Ledbury, Shropshire, 8 April; grounds of Selby Abbey, Selby, Yorks, 17 April; grounds of St Mary's Abbey, York, 18 April; Housesteads, Hadrian's Wall, Northumberland, 19 April; field by Caerlaverock Castle, Dumfries, Scotland, 20 April; Glencaple, near Dumfries, Scotland, 20 April; near Penrith, Cumberland, 21 April; Deanery Garden, Ely Cathedral, Isle of Ely, 23 April.

The thalli of this fungus were somewhat irregularly, even angularly peltate, sometimes strongly lobed, and were appressed to the surface of the substratum. Four to eight main axes up to 5 μ broad emerged at irregular intervals on the reproductive rudiment and these gave rise distally to richly branched, extensive rhizoids. A highly characteristic feature of the amber-coloured main body of the thallus, which varied from 28 to 50 μ in largest diam., was that the actual protoplast always appeared to be drawn away from the wall proper as though a slight plasmolysis had occurred. This was true of all specimens and evidently represented a normal condition. In a few instances several wall protuberances, probably discharge papillae, were found but no other features were observed. Although termed *Rhizophlyctis* sp. here, the form may well belong to *Phlyctorhiza* or even to the Hyphochytriales.

A second fungus of less doubtful affinities was found on insect wings in aquatic debris from the River Stour by Richborough Castle, Kent on 28 March. So few examples were seen that its method of development and precise manner of zoospore discharge could not be ascertained. The sporangia (Fig. 1 L) were spherical, 30–54 μ diam. and noteworthy in the possession of a very distinctly roughened, amber-coloured outer wall resembling in this respect *Rhizophlyctis ingoldii*. At least three, probably more, prominent, 10–13 μ diam. discharge papillae were scattered over the surface.

One to three rhizoidal trunks 5–7 μ broad arose from the base and gave rise distally to a much branched system of rhizoids. Zoospore discharge took place through one to three broad pores left upon the deliquescence of the papillae. Actual discharge of the zoospores, however, was not witnessed. From the great numbers left in the vicinity of the pores and within the sporangia it was evident that these were colourless, very minute, approximately 2·5 μ diam., bore a very inconspicuous globule, a more evident nuclear cap, and a long posterior flagellum.

Two other types deserve mention here. The first of these, found on grass bait in soil from a field near Orsett, Essex, 30 March, was characterized by somewhat pyriform sporangia, 30–50 × 20–25 μ, which bore a single broad discharge tube 10 μ diam. Its contents were orange-coloured. One, sometimes two, rhizoidal axes arose from the basal region. No spore discharge was observed.

The second fungus strongly resembled an unnamed *Rhizophlyctis*, possibly a form of *R. rosea*, found in Cuba and figured by Sparrow (1952, fig. G). It had amber-coloured, essentially spherical sporangia which were

rendered markedly stellate by reason of the production of numerous discharge tubes. It was recovered on cellophane from soil collected in a field near Bishop's Stortford, 30 March; on grass, from near Ledbury, Shropshire, 8 April; and on cellophane from the Cathedral Close, Lincoln, 17 April; on cellophane from the grounds of St Mary's Abbey, York, 18 April and from near Crooklands, Westmorland, 21 April.

Although these may be simply forms of *R. rosea*, they seemed sufficiently distinct to warrant special mention.

Cladochytrium tenue Nowakowski. Grass leaf, garden pool, 'Byron's Lodge', Grantchester, Cambs, 10 April.

Physoderma menyanthis de Bary. On *Menyanthes trifoliata*, Black Ditch, Cavenham Heath, Suffolk, 8 March.

Through the kind efforts of Dr N. F. Robertson of the Botany School, Cambridge, overwintered material of the resting spores of this species was obtained. From an earlier study of this species at this same site (Sparrow, 1946) there was an element of doubt as to whether the resting spores germinated by the circumsessile dehiscence of a cap or by mere fracturing of the wall by the developing sporangium. A study of this process in the new material clearly indicated that fracturing of the wall and not dehiscence of a cap was the rule.

Chytridium confervae (Wille) Minden. Parasitic on *Tribonema* sp., pool in Black Ditch, Cavenham Heath, Suffolk, 8 March. Abundant.

Chytridium lagenula Braun. Parasitic on *Tribonema* sp., pool, Black Ditch, Cavenham Heath, Suffolk, 8 March.

Chytriomyces hyalina Karling. On chitin. Soil, Cavenham Heath, Suffolk, 8 March; River Stour by Richborough Castle, Kent, 28 March.

Monoblepharidales

Gonapodya polymorpha Thaxter. Twigs. Dinas Powis, near Cardiff, Wales (coll. Perrott), 5 April; rear of Leys School, Cambridge, 21 May. Abundant gametangial formation as described by Johns & Benjamin (1954) was noted.

The following species of *Monoblepharis* were found in material kindly collected for me by Mrs Perrott from Dinas Powis, near Cardiff, Wales, 5 April: *M. sphaerica* Cornu, *M. hypogyna* Perrott, *M. polymorpha* Cornu, *M. insignis* var. *minor* Perrott, *M. bullata* Perrott.

Blastocladiales

Catenaria anguillulae Dang. Snake-skin. Soil, 17 Barton Road, Cambridge, 21 February; Deanery Garden, Ely, Isle of Ely, 23 April.

Another strongly polycentric form on snake-skin bait was recovered in soils from following localities: Fawnhope, near Hereford, 8 April; moat of Caerlaverock Castle, Dumfries, Scotland, 20 April; Ambleside, Westmorland, 21 April; near Crooklands, Westmorland, 21 April. It closely resembled the fungus figured by Karling (1949, fig. 16) as representing the resting stage of *Polychytrium aggregatum*. Since no zoospores were found

in any of these fungi their relationships remain conjectural. They seem most to resemble a species of *Catenaria* or even of *Hyphochytrium*.

Blastocladia globosa Kanouse. *Aesculus* twigs. Peterhouse Ditch, Cambridge, 11 March.

B. glomerata Sparrow. *Aesculus* twigs. Peterhouse Ditch, Cambridge, 11 March. Only resting spore plants were found.

Saprolegniales

Leptolegniella keratinophilum Huneycutt. On snake-skin bait. *Sphagnum* debris, Black Ditch, Cavenham Heath, Suffolk, 8 March. Only the highly characteristic sporangial stage was found.

Aphanomyces stellatus de Bary. On chitinous substrata. Vegetable debris Black Ditch, Cavenham Heath, Suffolk, 8 March.

Thraustotheca clavata (de Bary) Humphrey. Hemp-seed bait. Soil, Abergavenny, Wales, 11 April.

Brevilegnia linearis Coker & Braxton. Hemp-seed bait. Soil, rear of Botany School, Cambridge, 29 February; snake-skin bait, Cathedral Close, Canterbury, Kent, 27 March.

Brevilegnia sp. Human hair. Soil, St Botolph's churchyard, Cambridge, 7 March; garden, Abbots Barton Hotel, Canterbury, Kent, 29 March; woods near Harlow, Essex, 20 March; Lower Slaughter, Glos, 7 April; Stanton, Glos, 8 April; Ross-on-Wye, Herefordshire, 9 April; one mile south of Ely, Isle of Ely, 10 April; St Mary's Abbey grounds, York, 18 April; Ambleside, Westmorland, 21 April; Deanery Garden, Ely, Isle of Ely, 23 April.

This remarkable little fungus, which seems widespread in its occurrence, is deserving of a comprehensive morphological and physiological study.

It appears on the substratum very slowly, several weeks after a wave of invasion by the ubiquitous *Rhizophydium keratinophilum* has subsided.

The sporangia resemble those of *Brevilegnia linearis* Coker & Braxton and *B. minutandra* Höhnk in having the encysted zoospores borne for the most part in a single row in the sporangium. It appears, however, to be a much more delicate form than either of these. Thus, the hyphae are 6 μ diam., the sporangia 97 μ long by 6–12 μ wide, and the encysted zoospores are 4·5–6·5 μ diam. In *B. linearis* the hyphae are 8–14 μ diam. and the spores 8–20 by 15–60 μ, whereas in *B. minutandra* these structures (hyphae) are 7–19 μ diam., sporangia 400 μ to 2·4 mm. long by 7–19 μ diam. and the encysted zoospores 7–19 μ diam. and up to 85 μ long.

The absence of sex organs prevents further significant comparison with related species. It is distinctive in being strongly keratinophilic, no other types of bait being attacked, and in its delicacy. Further studies will undoubtedly show it to be an undescribed species.

In one sample from Housesteads, Hadrian's Wall, Northumberland, 19 April, and from near Crooklands, Westmorland, 27 April, another strongly keratinophilic form appeared on hair, which was wholly unrelated to the saprolegniaceous one. Since only partial data on its structure were obtained, many aspects of it need further study. In contrast to other hair inhabitants, this one swelled the substratum locally and formed chambers

produced around the main shaft of the hair (Fig. 2D–G). These thalli were, so far as could be ascertained, devoid of rhizoids and at maturity were, converted into olpidioid zoosporangia. These were predominantly ellipsoidal and 34–42 × 17–24 μ, occasionally spherical and 20 μ diam., and bore a single broad conical discharge tube, 7–10 μ long by 5 μ at the base. At maturity, the zoospores initiated movement within the sporangia and milled around until the deliquescence of the tip of the discharge tube when they were ejected to the outside medium (Fig. 2G). Here, the thin elongate bodies became more rounded and, describing a helical path, swam away. Neither in their shape, contents (lacking an oil globule), or their type of movement did they resemble chytrid zoospores. A single posterior flagellum was definitely observed and there was some indication that there was an anterior one as well. However, this biflagellation could not be confirmed with certainty. Furthermore, so few sporangia discharged that staining procedures to determine the number of flagella could not be employed. A few spherical resting spores about 20 μ in diameter with their outer walls covered by straight slender spines 3 μ long were also found (Fig. 2H). No companion cells were observed.

If, as seems probable, this is a biflagellate form, it is most certainly a member of the Olpidiopsidaceae. If, on the other hand, it is found to be uniflagellate, it belongs in *Olpidium*. Only further observations on this interesting keratinophilic form will determine its true affinities.

Leptomitales

Apodachlya brachynema (Hild.) Prings. On hemp-seed bait. Debris, Black Ditch, Cavenham Heath, Suffolk, 8 March; twigs, Jesus Ditch, Cambridge, 11 March; twigs, ditch, rear of Leys School, Cambridge, 27 May.

Apodachlya pyrifera Zopf. On twigs of *Aesculus*, Peterhouse Ditch, Cambridge, 11 March.

Lagenidiales

Olpidiopsis aphanomycis Cornu. In hyphae of *Aphanomyces* sp. on snakeskin. Soil, Housesteads, Hadrian's Wall, Northumberland, 21 April; sphagnum bog, Randy Pike, Hawkeshead, Lancs (coll. W. J. G. Lund), 21 April.

Olpidiopsis pythii (Butler) Karling. Parasitic on *Pythium* sp. Soil, St Botolph's churchyard, Cambridge, 7 March; Black Ditch, Cavenham Heath, Suffolk, 8 March; soil, overlying *Corstopitum* (Corbridge), Northumberland, 19 April; Housesteads, Hadrian's Wall, Northumberland, 19 April.

Olpidiopsis sp. In vegetative cells of *Mougeotia* sp., Black Ditch, Cavenham Heath, Suffolk, 8 March.

Whether this fungus is referable to *O. fibrillosa* de Wild. or *O. appendiculata* de Wild. cannot be ascertained. Its empty sporangia which rested in unhypertrophied cells of the host were 40 × 15 μ and bore a single, short discharge tube. The resting spores were spherical, 15 μ diam., and their outer walls were densely covered with slender tapering spines 10 μ long.

In contrast to the sporangia, the resting spores caused some hypertrophy of the infected cell. No companion cell was seen, although if these were small it could be easily hidden in some views by the resting spore.

Lagenidium humanum Karling. On insect wing, in algal debris, Orsett, Essex, 30 March. Although occurring on chitinous material rather than human skin where it was first found, the thallus and sporangial stage correspond closely with those of Karling's fungus.

Peronosporales

Zoophagus insidians Sommer. Predaceous on rotifers, Jesus Ditch, Cambridge, 11 March.

Pythiogeton uniforme Lund. Twigs, in ditch, rear of Leys School, Cambridge, 21 May.

Phytophthora gonapodyides (Petersen) Buismann. Twigs, Jesus Ditch, Cambridge. 11 March.

It is a pleasure to acknowledge my indebtedness to Prof. G. E. Briggs for the use of the facilities of the Botany School, and to Drs S. D. Garrett and N. F. Robertson for their many courtesies during my stay in Cambridge. It is also a genuine pleasure to thank Mrs Elizabeth Perrott, University College of North Staffordshire, for generously collecting for me submerged twigs in Wales. Finally, the warm welcome accorded me by my botanical colleagues after more than twenty years absence from their country was most gratifying, indeed.

Acknowledgement is also made to the Executive Board of the Horace H. Rackham School of Graduate Studies for a grant to make this research possible.

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BRITISH RECORDS, 14–16

14. **Pleurage fimbriata** (Bayer) comb.nov. (*Sordaria fimbriata* Bayer, A. *Acta Soc. Sci. Nat. Moraviae*, **1**, p. 71, 1924). This species developed on dung pellets of domestic rabbit obtained from the London School of Hygiene and Tropical Medicine and on similar pellets from Woolwich Polytechnic. Pure cultures were established on rabbit dung agar. The mycelium is nearly black on the surface of the medium, but grey and fluffy above. The olivaceous to black perithecia measure about 300 × 600 μ.

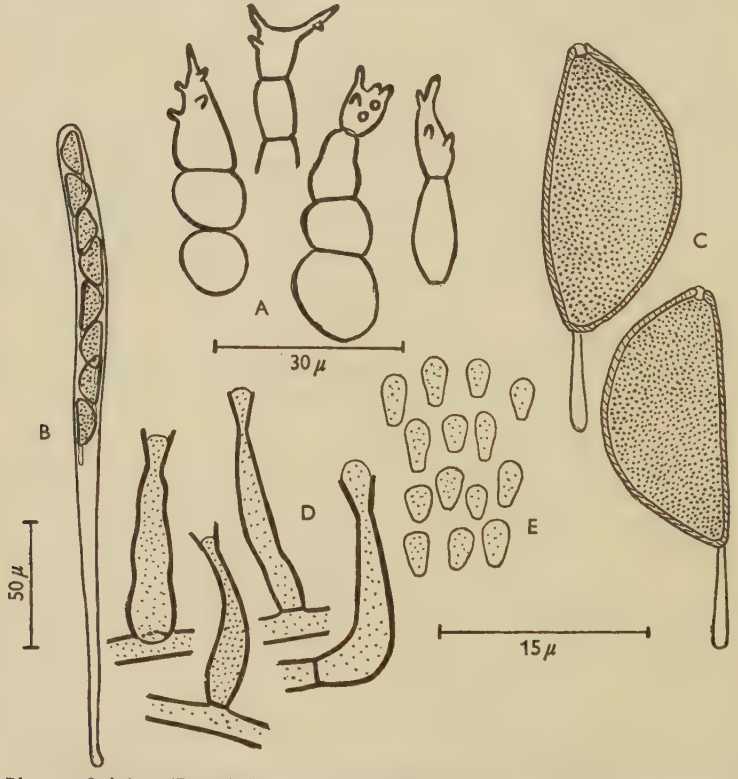

Fig. 1. *Pleurage fimbriata* (Bayer) Page. A, hairs from the neck of the perithecia; B, ascus; C, ascospores; D, phialides; E, microconidia. (C, D, and E same scale.)

The semi-transparent wall has numerous multicellular hairs, those on the neck having characteristic outgrowths from the terminal cell (Fig. 1 A). The asci are cylindrical, each with eight uniseriate spores. The ascospores are blackish 8·5–10 × 18·5–22 μ, flattened on one side and with a conspicuous apical germ-pore. The basal cellular appendage is small (about 12 μ long), straight and widens gradually from its attachment towards

its free end. In culture micro-conidia (phialospores) are produced abundantly. Each is somewhat clavate, a form determined largely by the funnel-shaped apex of the phialide.

The fungus agrees so closely with Bayer's figures of *Sordaria fimbriata* that there can be little doubt of its identification. It is, however, clearly very close to *Pleurage inaequalis* (Cain) Moreau. Dr Cain kindly sent me a culture of this fungus, so it has been possible to make a close comparison of the two species. The spores of both are so alike that it was thought at first that Cain's species was merely a 4-spored variety of *S. fimbriata*. However, there are a number of striking differences. In cultures of *P. inaequalis* the perithecia are much more densely aggregated and much smaller (150–350 μ) than those of *S. fimbriata*. Further in *P. inaequalis* the perithecial hairs are represented by single globose cells, which have outgrowths it is true, but not quite like those of the terminal cells of the hairs in *S. fimbriata*. Finally in cultures of *P. inaequalis* no microconidia are formed.

If C. Moreau's treatment of the Sordariaceae is to be followed, a new combination is necessary and so *Pleurage fimbriata* (Bayer) Page is proposed. Probably *Bombardia lunata* Zickler is a synonym. Herb. I.M.I. 68749, 68750.

WINIFRED PAGE

15. **Pleurage glutinans** (Cain) Moreau (Les *genres* Sordaria *et* Pleurage *Encyclopédie Mycologique*, **25**, p. 237, 1953). This species occurred on old rabbit pellets from Blakeney Point, Norfolk, and on the same substratum from Thorndon Park, near Brentwood, Essex. It was grown in pure culture on rabbit dung agar with filter-paper added. No microconidia were formed. Herb. I.M.I. 68751.

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16. **Sordaria barbata** Hansen (*Vidensk. Medd. dansk naturh. Foren. Kbh.* p. 57, 1876). This species, with its highly characteristic spores, developed on rabbit pellets from Thorndean Park near Brentwood, Essex. It was isolated and grown in pure culture on rabbit dung agar with filter-paper added. No microconidia were produced. Herb. I.M.I. 63849.

Like other species of *Sordaria* cellular appendages are absent from the spore. This species is sharply distinguished from others by the size and shape of the spore and by the presence of a fringe of fine mucilaginous projections looking somewhat like the cilia on a *Paramecium*. This fringe of projections is difficult to see except in freshly discharged spores. Another rather striking feature is that the maturing spore passes from golden brown to near black. There is no trace of the transient green pigment so frequently seen in developing sordariaceous spores.

WINIFRED PAGE

REVIEWS

Index of Fungi. Petrak's Lists 1920–1939. *Cumulative Index. Part 1 comprising the Index of Generic Names and the Index of Specific Epithets.* Pp. 1–168, 1956. 20*s*. *Part 2 comprising Host Index, Index of Host Genera, and Other Substrata Index.* Pp. 169–307. 20*s*. (Commonwealth Mycological Institute, Kew, Surrey.)

These two valuable indexes make more easily accessible the data contained in Petrak's Lists (see these *Transactions*, **36**, p. 77) and their usefulness will extend far beyond the herbarium.

Revision der zu Gloeosporium *gestellten Pilze.* By J. A. von Arx. Verhandelingen de Koninklijke Nederlandse Akademie van Wetenschappen, afd. Natuurkunde 51, No. 3, pp. 153, 40 figures. (N.V. Noord-Hollandsche Uitgevers Maatschappij, Amsterdam. 1957.) Price: 12 guilders.

This important work by a well-known authority on Pyrenomycetes and their conidial states is arranged in two sections. First is a monographic study of the Melanconiaceous genera having unicellular hyaline conidia, with a key to the twenty-nine genera recognized. Two new genera are proposed, viz. *Rhynchosporina* von Arx, based on *Gloeosporium meinersii* Sprague, on grasses in U.S.A., and *Melanophora* von Arx, based on *Gloeosporium crataegi* Dearn. & Barth. Twenty-one of the genera are illustrated by clear anatomical drawings such as one has come to associate with the author's work.

Mycologists have long realized that no distinction can be drawn between *Gloeosporium* sensu Saccardo and *Colletotrichum* Corda. The latter name is adopted here, for *Gloeosporium* was originally proposed by Desmazières and Montagne as a monotypic genus based on what is now called *Marssonina castagnei* (Desm. & Mont.) Magnus, i.e. a fungus with 2-celled conidia. *Marssonina* Magnus 1906 is proposed for conservation against *Gloeosporium* Desm. & Mont. 1849, to avoid the large number of name changes which would result from reviving the latter.

The second and longer part of the work is an alphabetical annotated list of the 735 specific names hitherto published in *Gloeosporium*. Specimens assigned to 641 of these have been studied and reclassified, a process which has led the author to propose 125 new combinations and to make extensive relegation to synonymy. Thus 288 specific names in *Gloeosporium* are considered to have been based on the conidial state of *Glomerella cingulata* (Stonem.) Spauld & v. Schr. British readers will find many familiar species redisposed but they should realise that several of the changes were proposed long ago by earlier taxonomists and are in current use in continental countries. Many of the species dealt with are of considerable economic importance and this is accordingly a book which should be on the shelves of every practising plant pathologist.

R. W. G. Dennis

A Glossary of Mycology. By Walter H. Snell and Esther A. Dick, with illustrations by Henry A. C. Jackson. (Cambridge, Mass.: Harvard University Press; London: Oxford University Press. 1957.) Pp. xiv + 171, 15 plates (191 figures). Price: 40*s*.

Going bald-headed for this work, the first thing one may notice is the number of synonyms for hairy, with their diagrams (pl. IV). But the somewhat stiff-looking drawings are not a main part of the book, though it is pleasing to record that 'oval' is here correctly

equated with 'ovate' (pl. XIII) and not 'elliptical' (contrast Ainsworth & Bisby, *A Dictionary of the Fungi*, fig. 113).

The avowed purpose of the authors is to provide definitions of terms relating to fungi or in general literature of interest to mycologists: technical, common or popular, vernacular and obsolete; terms used in the field of medical mycology and antibiotics: names of the originators of the terms; folklore terms and colour terms. The definitions are easy to read and to understand, though quite clearly for the mycologist and not for the lay public.

Any glossary of words mainly scientific could aim to teach the reader what he did not know before, or to select preferred definitions (and hence meanings) out of the available welter of possibilities, or, thirdly, to offer a review of all the different usages known to the compilers. The third aim is that of the dictionary maker, the first, that of the encyclopaedist, while the second more didactic role is usually disclaimed outside the classroom. Yet it inevitably tinges the outlook of even the most would-be impartial, and probably the best way of dealing with this tendency is to bring it into the open as much as possible. Thus the compilers here do at least use the qualification 'obs.', though surprisingly rarely, so that at first it is easy to overlook it. There are two ways in which a word can become effectively obsolete: by being part of the report of an observational error or of the apparatus of a defunct theory, or by mere disuse. No doubt the corpse of 'hadromycosis', as of 'klendusity' (not given), twitches from time to time, but if not truly obsolete, such words at least deserve the mark ⊙—'I have gone home'. There is very little overlap between this Glossary (S and D) and the Dictionary mentioned above (A and B). Of twenty entries taken at random from S and D only six were covered by A and B. Of twenty entries in the latter, fifteen were generic names and two were within the encyclopaedic longer articles; the functions of the two works are substantially different and they are largely complementary.

Some oddments may be noted. The inclusion of names of some substances, chiefly antibiotics, is a doubtful asset. Information of this kind is overtaken by discovery at a very rapid rate, and one would naturally tend to look for it elsewhere. 'Fictitious = false' (*tout court*). This is not so. In biology, particularly, 'false' bears the second meaning = 'pseudo-', 'not what it appears to be', whereas 'fictitious' must always mean 'described, but not existing'. 'Disease': though touching lightly on plant pathology, a subject that used to attract mycologists before the discovery of penicillin, the compilers attempt no definition of this word, an omission that may perhaps disappoint medical mycologists also. Perhaps they knew the difficulties.

A situation that needs amending in the literature generally is the one that may be parodied. Textbook or Flora: 'In *Golbonium frustrax* the surface is minutely smogloid'. Dictionary: 'smogloid—of a surface, minutely broken up in a special manner, as in *Golbonium frustrax*.' This circularity is the despair of non-taxonomists, because collectors and describers are nearly always too impatient to say what they see, taking refuge in descriptive terms of woolly significance instead of describing appearances in comparative or objectively metaphorical language—which should be possible, even in Latin. Glossaries can do a brave work in remedying the consequent obfuscation and one strongly welcomes a definition here of 'shagreen'—'minutely cobblestoned', even if it in turn is doomed to obscurity by the progress of road improvement. Indeed it reassures one of the validity of a first impression, that this glossary should be in every library used by mycologists.

E. R. WALLACE

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